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THE RESPIRATORY AND RIPENING BEHAVIOUR OF THE TOMATO FRUIT ON THE PLANT[1]

BY K. A. CLENDENNING[2]

Abstract

The respiratory behaviour of tomato fruits during their growth and ripening on the plant has been studied in relation to the season and light intensity during growth. Growth was found to be associated with an absolute increase in respiration rate. Fruits of all populations showed a marked rise and fall in respiration rate during ripening. This senescent drift was confirmed in detail by following the respiration of individual fruits ripening while still attached to the plant. Although exhibiting the usual senescent drift as they changed colour, fruits grown in summer cloth house plots showed a consistently lower rate of respiration than that observed in all other populations. It also has been shown that the chief path of gaseous exchange of the mature attached fruit is localized at the stem end.

The present investigation was undertaken as one aspect of a study of the metabolism of the tomato fruit in development and storage. Information has been published recently (3) on the respiratory and ripening behaviour of the stored fruit in relation to the composition of its internal atmosphere. The present paper is concerned with the respiratory behaviour of the attached fruit in relation to the season and light intensity during growth. The area of free gaseous exchange also has been demonstrated to be at the stem end as in the stored fruit. Several biochemical aspects of the physiology of the tomato fruit in development and storage will be dealt with in subsequent reports.

Materials and Methods

The experimental material consisted of fruits of the Grand Rapids variety grown during 1938 at the Department of Botany, University of Toronto, and during 1938–1940 at the Department of Horticulture, Ontario Agricultural College. A comparison of populations grown at different seasons of the year necessarily involved a study of a series of greenhouse populations. The temperature was not allowed to drop below 60° F. but the atmospheric factors of the houses were not otherwise controlled.

[1] *Manuscript received November 18, 1941.*

Contribution from the Department of Horticulture, Ontario Agricultural College, Guelph, Ont., with the co-operation of the Department of Botany, University of Toronto, Toronto, Ont. This paper is based on part of a thesis submitted to the Graduate School of the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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During the summer of 1938, an ordinary field plot also was grown alongside a field cloth house plot in which the light intensity was reduced by 50% as measured by a Weston phototronic foot-candle meter. In the following summer, populations were grown simultaneously in a greenhouse bed, in an ordinary unshaded outdoor bed, and in a cloth house bed shaded to 50% light intensity. To simulate the soil conditions of the greenhouse population, the shaded and unshaded outdoor populations were grown in the same depth of standard soil as was used in the greenhouse; also, the outdoor beds were isolated from adjacent soil by a 12 in. layer of washed cinders.

Measurements of respiration rate were made by the Pettenkofer tube method, employing a continuous air flow of approximately 1500 cc. per hour. As a standard temperature, 12.5° C. was adopted for initial respiration rate determinations while 18.3° C. was used in studies of the carbon dioxide emission of attached fruits. The air stream was drawn through soda lime towers to remove atmospheric carbon dioxide. It then was bubbled through 18% potassium hydroxide to adjust its relative humidity to 85% before entering the individual respiration chambers.

It has been shown that the tomato fruit passes through a growth cycle consisting of a phase of cell division followed by a phase of cell enlargement. The former is of brief duration, persisting only for about one week from setting and apparently it results in the production of a cell population that is roughly uniform in number from fruit to fruit (4). The latter phase, a matter entirely of the enlargement of existing cells, persists until the mature green stage is reached, after which the fruit undergoes little change in volume or weight. It is therefore clear that the calculation of respiration rates on a fresh weight basis from the mature green stage onward through the ripening phase yields data that not only describe the changes in rate per unit of gross mass of living system but also provide a useful index to the changing activity of the individual fruit and even of the average cell. The same obviously cannot be said of the stages antecedent to maturity. At successive stages of the enlargement phase, unit fresh weight (e.g., 10 kg.) represents not only decreasing numbers of fruits but also proportionately decreasing numbers of cells. Hence respiration per unit fresh weight during the growth cycle reveals nothing of the changes in progress per fruit or per cell. Indeed, since these changes are sometimes in the opposite sense to the changes per unit fresh weight, the observer may be misled in matters of physiological interest by reasoning exclusively from data that involve the one method of expression alone. The data for the growth phases are accordingly presented here both on a unit fresh weight and on a unit fruit basis.

Experimental Results

Changes in Respiration Rate Associated with Growth

Earlier authors have agreed that at successive stages of growth, increasing size of the fruit is associated with a diminishing rate of respiration until a minimal rate is attained just prior to the onset of ripening (1, 5, 8). These

conclusions, however, were based on respiratory data calculated on a unit fresh weight basis.

To demonstrate the drift in respiration associated with the last four weeks of growth, the initial rate of respiration of fruits picked when "early growing green" (8), and "mature green", was determined individually on 12 fruits of each class. The average fresh weight of the early growing green class, picked at an age of three weeks, was 22.3 gm. while that of the mature green fruits, seven weeks old, was 83.4 gm. All these fruits were from a 1940 early summer greenhouse population. Immediately after detachment and cooling to the storage temperature, the early growing green fruits showed an average rate of respiration of 4.7 cc. carbon dioxide per fruit per day at 12.5° C. The mature green fruits on the other hand respired at an average rate of 9.6 cc. carbon dioxide per fruit per day under the same experimental conditions. Thus during the final four weeks of growth, there was approximately a 100% increase in rate of carbon dioxide emission per fruit. When calculated on a fresh weight basis, the same fruits showed the downward drift recorded by earlier authors; the early growing green fruits had an initial rate of 87.3 cc. carbon dioxide per 10 kg. fresh weight per hour while the mature green fruits had a rate of only 48.0 cc. per hour on the same fresh weight basis.

Changes in Respiration Rate Associated with Ripening

The respiratory behaviour of the tomato fruit ripening in storage is unique in that the expected senescent rise and fall in carbon dioxide output may be abolished completely by appropriate restriction of gaseous interchange at the stem scar area (3). Several authors have claimed that the tomato fruit always shows a marked rise and fall in respiration rate as it ripens on the plant, but for the most part their observations were limited to populations grown at one season of the year (1, 5, 7, 8).

The initial respiration rate of fruits picked when mature green, yellow orange, and full red has been determined in the course of the present work for populations grown at different seasons of the year and under different light conditions during the summer. In each population, it was evident that passage through the ripening colour change on the vine involved at first a rise in respiration to the yellow orange stage, followed by a decline as the fruits ripened to full red (Table I). The season in which the fruits were grown showed no clear effect on the extent of these respiratory changes. With the exception of the cloth house or midsummer 50% light plots, the data in fact conformed closely with those published by Walford (8) for respiratory changes during ripening in situ. The cloth house fruits showed a parallel rise and fall in respiration during ripening but their level of respiration was consistently lower.

In common with all previous studies of this kind on the tomato fruit, the above work was limited to determinations of the initial rate of respiration at successive stages of ripening. To obtain a more detailed picture of the general respiration sequence established by initial rate determinations, con-

TABLE I

THE MEAN INITIAL RESPIRATION RATE OF FRUITS OF SEVERAL POPULATIONS PICKED BEFORE, IN THE MIDST OF, AND AFTER THE RIPENING COLOUR CHANGE. RESPIRATION EXPRESSED AS CC. CARBON DIOXIDE PER 10 KG. PER HOUR

Populations	Mature green	Yellow orange	Full red
1939, spring greenhouse	47	114	71
1939, late summer greenhouse	49	76	48
1939, cloth house	40	54	29
1940, early summer greenhouse	49	97	83
1940, midwinter greenhouse	52	97	57

tinuous measurements of respiration were made on fruits ripening while actually attached to the plant (Fig. 1A, B, C).

It should be noted that the only study of this kind that had been attempted previously on fruits was that of the apple reported by Kidd and West (6). In that study, the respiration chamber was opaque so that the colour changes could not be observed and the fruit became detached at some unknown time in the course of their experiment.

In the present study, a tomato plant that had set six trusses of fruits was transferred to a storage room maintained at a temperature of 18.3° C. Large glass jars fitted with three hole rubber stoppers were used as individual respiration chambers for the attached fruits. The stoppers were pared to a depth of one-half inch. The central hole in each stopper was of such a size that it would fit snugly around the pedicel without damaging its tissues. To insert the pedicel, the stopper was slit from its outer edge to the central hole. By holding this incision open it was possible to place the pedicel in the hole and on closing the incision a fair seal was obtained by the pressure on the cut rubber faces. The other two holes of the stopper took the inlet and outlet tubes for the air stream. The stopper then was fitted snugly into a glass jar and was smeared carefully with vaseline until tests showed the respiration

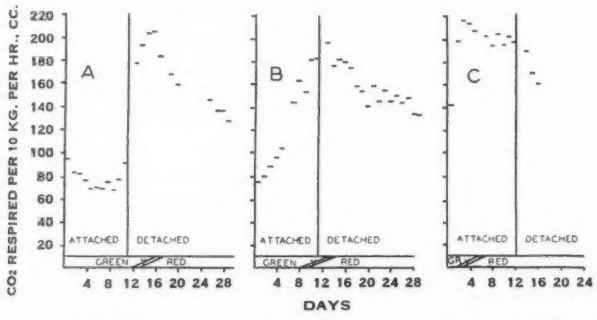

FIG. 1. *The respiration at 18.3° C. of the tomato fruit on the plant, with detachment at the onset (A), at the peak (B), and on the declining arm (C), of the respiratory climacteric.*

chamber to be airtight. Measurements of carbon dioxide output were taken by the usual continuous stream Pettenkofer method. Observations of ripening coloration were recorded daily. Finally the fruits were removed and weighed so as to allow calculation of the respiration data on a final fresh weight basis.

During the course of this experiment, the leaves of the plant turned yellow and after 12 days many of the leaves were brown. Because of this unhealthy condition of the leaves, the fruits in the respiration chambers were detached from the plant by severance of the pedicel (Fig. 1A, B, C), the respiration air stream being stopped only temporarily.

Fig. 1A, B, and C remove all doubt as to the reality of the climacteric rise in situ for the tomato fruit. The exact time of detachment in the present experiment is indicated and it is quite apparent that the act of isolation induces no significant change in the respiratory drift.

The respiration rate at 18.3° C. before the onset of the senescent rise approximated 70 cc. of carbon dioxide per 10 kg. per hour. The carbon dioxide output rose considerably before ripening coloration became evident externally. The appearance of orange colour in the outer tissues under these experimental conditions marked the attainment of highest respiratory activity. Subsequently the rate drifted downward until the fruits were dead ripe.

In agreement with Walford (8) and Kidd and West (6), it is clear from Fig. 1A, B, and C that the act of detachment does not interfere with the respiratory sequence which would have been exhibited by the fruit, had it remained on the plant. From this conclusion it should follow that the gaseous exchange at the stem end of the fruit which has been shown to be of such importance in the detached fruit cannot be influenced greatly by any partial plugging effected by the pedicel of the attached fruit. There is evidence that leaving a short piece of stem on the stored fruit results in a slight restriction of its gaseous exchange (2, 9) but the order of this restriction is by no means comparable to that observed when the stem scar area is waxed (2, 3). The drift in respiration rate of fruits in storage is essentially the same for those in which stems are removed as for those in which stems are attached (3). If the presence of the stem does not interfere greatly with gaseous exchange at the stem end of fruits either on the plant or in storage, the question arises as to the area through which the unobstructed diffusion of carbon dioxide and oxygen occurs. Do the gases from the fruit move through the pedicel or through other areas of the stem end? Or is the attached fruit characterized by a preponderance of cuticular diffusion in contrast with the detached fruit in storage?

By blocking diffusion at the stem scar area with wax, it has been shown elsewhere (2, 3), that the ripening of isolated mature green tomato fruits is seriously affected, the colour change always proceeding very slowly and unevenly. This alteration in ripening behaviour has also been shown to be a result of the changes induced in the fruit's internal atmosphere and is of

sufficient reliability to be used as an indication of the effectiveness of a particular waxing treatment in blocking gaseous exchange.

Copious application of vaseline or melted paraffin wax (175° F.) has been used previously with equal success in demonstrating that the gaseous exchange of the isolated fruit is localized principally at the stem scar area. In a study designed to indicate the path of gaseous exchange of the attached fruit these two substances have been used to block diffusion at different areas.

Five lots of attached mature green fruits were prepared in the following manner for observations of ripening behaviour:

Lot 1. Calyx left attached and no other treatment given.

Lot 2. Calyx carefully removed, otherwise untreated.

Lot 3. Calyx removed and melted paraffin wax (175° F.) applied to the area of the fruit ordinarily covered by the calyx, to the parts of the pedicel immediately adjacent to the receptacle, and to the region of the receptacle itself in the angle between pedicel and fruit.

Lot 4. Calyx removed and the pedicels heavily coated with vaseline from a few millimetres above the receptacle to the point at which the pedicel branches from the truss.

Lot 5. Calyx removed and the skin of the fruit heavily coated with vaseline, leaving the pedicel and calyx area untreated.

The ripening behaviour of Lots 1, 2, and 4 was normal, all fruits attaining a full red colour within seven days from the time ripening commenced. The ripening of Lot 5 was also not inhibited but its treatment resulted in the appearance of brown blotches on the skin. The fruits that had been waxed at the stem end (Lot 3) on the other hand showed a marked inhibition of ripening rate, never attaining more than a pale orange colour. Twenty-one days after the first appearance of yellowing, all fruits of Lot 3 were not more than pale orange colour, although quite as soft as overripe fruit. With but one exception, the fruits of this lot dropped to the ground by natural abscission. The final appearance of the one fruit of this lot remaining in a state of attachment for 60 days was of particular interest. When picked after remaining on the plant for 60 days after copious waxing at the stem end, this fruit was mostly pale orange in colour but showed typical symptoms of blotchy ripening over the locules. Green coloration still was evident on the shoulder. There also was evidence of surface pitting (3) over one locule. Like the other fruits of this lot, softening progressed to a marked extent despite the inhibited ripening colour changes. It should be recalled that this physiological anomaly for which the term "premature softening" has been proposed has been observed frequently in fruits stored with stem scars waxed (3).

Thus the only treatment of attached fruits that caused an inhibition of ripening coloration was the application of wax to the stem end or calyx area. The area of free gaseous diffusion in the attached tomato fruit therefore must be localized at the stem end just as it is in the unwaxed fruit in storage. It further appears that in the attached fruit, the unobstructed path of gaseous

exchange is through that part of the fruit ordinarily covered by the calyx, or through the immediately adjacent parts of the receptacle and pedicel.

The restriction of gaseous exchange at the stem end of the fruit and the resulting alteration of the fruit's internal atmosphere inevitably leads to a marked inhibition of the ripening rate and usually to an unevenness in the colour attained either on the vine or in storage. These characteristic accompaniments of obstructed gaseous exchange are also symptoms of the common physiological disorders "blotchy ripening" and "leather end". The possibility of unusual local tensions of oxygen and carbon dioxide in the fruit flesh contributing to the appearance of these developmental disorders appears sufficiently feasible to warrant experimental investigation.

Acknowledgments

In conclusion, the author wishes to express his appreciation to Professor G. H. Duff and to Dr. J. H. L. Truscott for their constructive criticisms of the experimentation and manuscript. The author is indebted to E. Roy Waygood who conducted the respiration studies on attached fruits.

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VEGETATIVE PROPAGATION OF CONIFERS

XII. EFFECTS OF MEDIA, TIME OF COLLECTION, AND INDOLYLACETIC ACID TREATMENT ON THE ROOTING OF WHITE PINE AND WHITE SPRUCE CUTTINGS[1]

By J. L. Farrar[2] and N. H. Grace[3]

Abstract

Semimonthly collections of white pine and white spruce cuttings were taken from July to October, 1939, and propagated in several media. Collections of both species were taken in late October to examine the effect of type of cutting and of planting in media involving different proportions of two sands and two different peats. Cuttings were dusted with a series of concentrations of indolylacetic acid in talc. The season of collection and the medium used for propagation were the factors of main importance. Phytohormone treatment failed to demonstrate appreciable effect, no difference in rooting response could be attributed to the kind of sand used, but there were indications that response increased with the amount of sedge peat in the medium.

Rooting of white pine cuttings collected in late August and propagated in a sedge peat medium was 62%, earlier and later collections gave substantially less rooting. Sand only and the sphagnum peat media were generally inferior to the sedge type of peat. At the optimum season of collection the sphagnum peat effected 50% rooting.

The late July collection of white spruce cuttings effected rooting of 90% of the plain cuttings when propagation occurred in a sedge peat medium. Low percentages rooted in sand or sphagnum peat media. Cuttings with a heel of old wood tended to be superior to plain cuttings in respect to survival and rooting.

Preliminary experiments with spring and early summer collections of both species resulted in slight rooting. Likewise, greenhouse propagations of dormant material gave very poor results.

An earlier communication dealt briefly with results of experiments on rooting cuttings of white pine (*Pinus strobus* L.) and white spruce (*Picea glauca* (Mœnch)) (6). This article deals with the same experiments in greater detail. The development of efficient methods for the vegetative propagation of economic native conifers is an important phase of a program of tree breeding. The investigations herein reported developed from a series of experiments in which cuttings of Norway spruce (*Picea Abies* (L.) Karst.) were used (2, 5–8, 11–19).

A number of other investigators recently have reported on the vegetative propagation of pines and spruces (1, 3, 4, 9, 10, 20–26, 28–30).

Experimental

The experiments on the propagation of white pine and white spruce cuttings involved consideration of the period of collection, media, type of cutting, and various chemical treatments. Most of the experiments were of factorial design, all were arranged with treatments replicated and groups randomized

[1] *Manuscript received February 6, 1942.*

Contribution from the Dominion Forest Service, Ottawa, and the Division of Biology and Agriculture, National Research Laboratories. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. N.R.C. No. 1043.

[2] *Pilot Officer, Royal Canadian Air Force. Formerly Forest Engineer, Dominion Forest Service, Ottawa, Canada.*

[3] *Biochemist, National Research Laboratories, Ottawa.*

in the propagating frames (27). Data were subjected to the analysis of variance procedure. Methods of collecting, treating and planting cuttings, the outdoor propagation frames, the various media, and the observations and measurements recorded have been described in earlier articles on the propagation of Norway spruce (7, 8, 17, 18).

The experiments herein described in detail were all initiated during the summer and autumn of 1939 at the Petawawa Forest Experiment Station, Chalk River, Ont. The four experiments dealing with white pine involved the use of 3200 cuttings and the four similar experiments with white spruce, 5480 cuttings. White pine cuttings for Experiments 1 and 2 were taken from trees 10 to 15 yr. of age. Only two branches were collected from any one tree. The terminal 5 to 10 cm. of second order twigs was used for cuttings. Cuttings for Experiments 3 and 4 were taken from the lower part of trees that were 16 yr. of age and somewhat larger than those mentioned above. All white spruce cuttings were taken from the lower part of trees 17 yr. of age. Cuttings were full length and ranged from 5 to 10 cm. in length. Those of Experiments 5 and 6 comprised cuttings with and without a heel of old wood. In Experiments 7 and 8 only full length plain cuttings were used. Experiments 1 to 4 with white pine and 5 to 8 with white spruce were propagated in the same media and frames as Experiments 1 to 4 of a paper dealing with Norway spruce (7).

White Pine

Experiment 1

Bimonthly collections were taken during July and August and planted in three media, namely, sand, sand mixed with one-third by volume of sphagnum peat, and sand with one-third volume of sedge peat. Each collection comprised groups of untreated cuttings and groups treated with powdered talc alone and containing 10, 100, and 1000 p.p.m. (parts of chemical to a million parts of the mixture by weight) of indolylacetic acid.

Experiment 2

The bimonthly collections of Experiment 1 were continued through September and October. Media were reduced to two by eliminating the sphagnum peat mixture.

Experiment 3

One collection of cuttings was taken and planted late October. There were three types of cuttings, namely, Type 1, first order terminal twigs; Type 2, second order terminals, and Type 3, second order laterals. These types have been described and illustrated in an earlier article (8). Cuttings were planted in the two peat media described under Experiment 1.

Experiment 4

Cuttings were collected late October and planted in 28 different media in compartments 1×3.5 ft. in size. The media comprised three grades of sand and various proportions of sedge peat and sphagnum peat, and have already been described in detail (7).

White Spruce

Experiments 5 and 6

These experiments were similar to Experiments 1 and 2 in respect to collections, media, and chemical treatments. Both plain cuttings and cuttings with a heel of old wood were used.

Experiment 7

Six types of cuttings were planted in the two peat media described under Experiment 1. These six types of cuttings already have been described in an experiment dealing with Norway spruce (8). The experiment comprised one late October collection of cuttings.

Experiment 8

A late October collection of full length plain cuttings was planted in the 28 media used for Experiment 4 (7).

Cuttings of all these experiments were removed for counts and measurements in September, 1940.

A number of earlier experiments with both white pine and white spruce were initiated during 1938 and the spring of 1939. These experiments dealt with both greenhouse and outdoor plantings in sand and sand with sphagnum peat. Results of these experiments will not be given in detail but will be briefly summarized.

Results

White Pine

Table I indicates the effect of season of collection and propagation medium on the responses of white pine cuttings of Experiments 1 and 2. Since the phytohormone treatment failed to have any significant effect, the data are averages over all treatments with indolylacetic acid, excepting the 1000 p.p.m. level in Experiment 1 which was somewhat injurious. In the sedge peat medium survival and rooting were low for the mid-July collection but increased rapidly. The best rooting, 62%, was obtained with the late August collection. Thereafter a general decline in rooting was noted. However, survival was maintained and development of new growth was somewhat greater. During September and October rooting averaged a little better than 40%. The development of new growth and the length of roots was substantially greater in the sedge peat medium than in sand. Most of the cuttings had only one root, though a few had two or three roots. In sand only there was slight rooting and survival in the first four collections (Experiment 1), hence the data were not included in the table. The four later collections in sand (Experiment 2) indicated substantial survival, but rooting was low, ranging between 12 and 22%. In the sphagnum peat medium survival and rooting were not as good as in sedge peat.

Cuttings of Experiment 3 survived to the extent of about 60% in both media but average rooting was low. Approximately 21% of the cuttings in sphagnum peat rooted, against 10% in the sedge peat medium. Type of cuttings had no appreciable effect on rooting in sedge peat. In the sphagnum

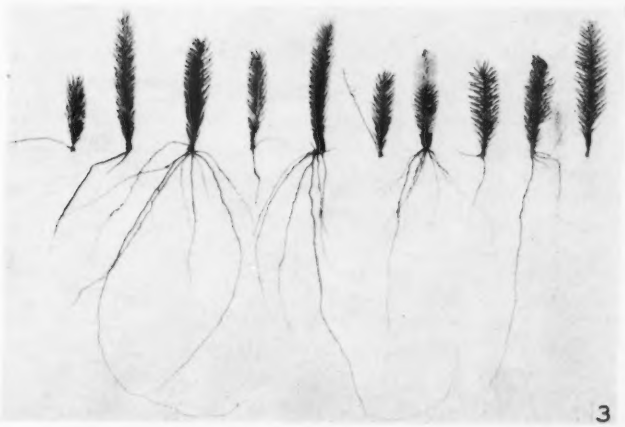

FIG. 1. *Miscellaneous group of white pine cuttings showing size and character of roots, callus and new growth.*

FIG. 2. *Unit of 10 white spruce cuttings with a heel of old wood propagated in sedge peat medium.*

FIG. 3. *Surviving cuttings of unit of 10 plain white spruce cuttings (without a heel of old wood) propagated in sedge peat medium.*

TABLE 1

ROOTING RESPONSES OF WHITE PINE CUTTINGS

	Date of collection															
	Experiment 1								Experiment 2							
	July 18		Aug. 1		Aug. 15		Aug. 29		Sept. 18		Oct. 2		Oct. 16		Oct. 30	
	Sedge peat	Sphagnum peat	Sedge peat	Sphagnum peat	Sedge peat	Sphagnum peat	Sedge peat	Sphagnum peat	Sedge peat	Sand	Sedge peat	Sand	Sedge peat	Sand	Sedge peat	Sand
Number of surviving cuttings, %	25	0	57	37	100	85	92	47	80	51	86	66	85	68	89	88
Number of rooted cuttings, %	22	0	32	10	57	50	62	7	44	12	47	19	44	14	36	22
Number of cuttings rooted as percentage of those surviving	90	—	56	27	57	59	68	16	55	24	55	29	52	21	41	25
Number of roots per rooted cutting	1.1	—	1.0	1.5	1.3	1.6	1.2	1.0	1.2	1.8	1.2	1.5	1.3	1.1	1.2	1.3
Length of roots per cutting rooted, mm.	*								146	93	149	86	157	50	162	96
Mean root length, mm.	*								119	51	123	58	123	43	129	76
Number of cuttings with new growth, %	17	0	30	2	40	20	57	0	60	4	67	22	64	16	80	43
Number of cuttings with new growth as a percentage of those surviving	70	—	52	67	40	24	62	—	75	8	78	33	75	24	90	49
Number of rooted cuttings with new growth as percentage of those rooted	67	—	61	25	43	30	76	—	77	25	79	53	76	36	100	59
Number of rooted cuttings with new growth as a percentage of those with new growth	86	—	67	100	81	75	83	—	57	75	55	45	48	31	45	30

* *Many of the roots were broken on removal and therefore data for lengths are not given.*

TABLE II

ROOTING RESPONSES OF WHITE SPRUCE CUTTINGS IN A SEDGE PEAT MEDIUM

	Kind of cutting	Date of collection															
		Experiment 5								Experiment 6							
		July 13		July 27		Aug. 10		Aug. 24		Sept. 16		Sept. 30		Oct. 14		Oct. 28	
Number of surviving cuttings, %	Plain	46		94		56		56		24		45		26		24	
	Heels		94		92		76		76		44		26		44		21
Number of rooted cuttings, %	Plain	38		90		44		32		16		37		23		15	
	Heels		76		84		62		56		28		21		23		14
Number of cuttings rooted as a percentage of those surviving	Plain	83		96		79		57		67		82		88		63	
	Heels		81		91		82		74		64		81		52		67
Number of roots per rooted cutting	Plain	2.9		3.4		3.6		3.1		2.0		2.6		1.9		2.1	
	Heels		2.9		3.9		3.3		2.7		2.5		2.2		2.0		2.0
Length of roots per cutting rooted, mm.	Plain	259		233		425		242		94		183		128		128	
	Heels		254		492		347		253		205		119		94		139
Mean root length, mm.	Plain	91		68		117		79		47		71		68		60	
	Heels		87		127		104		94		81		54		48		69
Number of cuttings with new growth, %	Plain	10		14		6		16		2		10		4		8	
	Heels		8		2		2		6		5		7		7		6

peat 15% of the cuttings of Types 1 and 3 rooted, against 33% of Type 2. Cuttings of Type 1 developed more new growth than those of Types 2 and 3. The respective percentages were 53, 32, and 27%, averaged over both media. Whereas 44% of the cuttings planted in sedge peat had new growth, the value was 31% for sphagnum peat plantings.

Survival and rooting of cuttings of Experiment 4 were not affected significantly by the texture of the sand nor by the proportion of peat. However, there were differences between the average values for sand only and the two different types of peat. In sand, survival was 54% and rooting, 5%; in sedge peat the values were, respectively, 71 and 16%; and in sphagnum peat, 65 and 15%. New growth was developed to the extent of 15% in sand, 50% in sedge peat, and 31% in sphagnum peat. Fig. 1 shows a miscellaneous group of cuttings from this experiment, depicting size and character of root, callus formation, and new growth.

None of the authors' earlier experiments with white pine resulted in appreciable rooting. While results in the greenhouse and outdoors in sand were negative, a midsummer collection in 1938 planted in sphagnum peat and sand resulted in about 4% rooting. Greenhouse experiments with summer collections of cuttings propagated in sand and sedge peat and sand using bottom heat (25) speedily resulted in 100% mortality.

White Spruce

All cuttings of Experiment 5 planted in sand died, and less than 1% of those of Experiment 6 were rooted. Sphagnum peat plantings in Experiment 5 resulted in approximately 2% rooted cuttings. In consequence the data given in Table II are limited to the results from cuttings planted in the sedge peat medium. The data are averages over all indolylacetic acid treatments. Date of collection and type of cuttings both had a marked bearing on the responses. Rooting reached a peak of 84 to 90% in the late July collection. Succeeding collections indicated a gradually deteriorating response. Cuttings with a heel were, on the average, substantially better than plain cuttings. When rooting was at its peak, however, plain cuttings rooted fully as well or better than those with a heel. Length of root attained a maximum of 492 mm. per rooted cutting with a heel for the late July collection, and 425 mm. for plain cuttings of the mid-August collection. Root length declined markedly in later collections. New growth development was rather poor, and plain cuttings appeared superior to those with a heel in this respect. Groups of white spruce cuttings from the July 27 collection, plain and with a heel of old wood, are shown in Figs. 2 and 3.

None of the indolylacetic acid treatments showed a significant effect on rooting or survival. In Experiment 5 indolylacetic acid affected the number of roots per rooted cutting with new growth. The average values were 2.5, 2.7, 3.1, 3.1, and 3.5 roots, respectively, for untreated, talc treated, and treated with 10, 100, and 1000 p.p.m. of indolylacetic acid in talc; the necessary difference for the 5% level of significance was 0.16.

There was 100% mortality of the cuttings of Experiment 7 planted in sphagnum peat. Survival and rooting in sedge peat were poor and it was impossible to demonstrate significant variation in the responses of the six types of cuttings, though certain differences were suggested. Survival of first order terminal cuttings (Type 1) was 5% and rooting, 3%, whereas the corresponding average values for the other five types were 22 and 8%. The best rooting, 16%, was shown by second order terminal cuttings (Type 2). Survival of shortened first order terminal cuttings (Type 6) was about 25% with 3% rooting.

Survival and rooting of cuttings of Experiment 8 were low for plantings in the sedge peat medium, whereas there was 100% mortality of cuttings planted in sand or sphagnum peat. Rooting of 12% occurred in the medium containing equal volumes of sedge peat and sand. The response was poorer for media with a lower proportion of sedge peat.

The undesignated experiments with white spruce resulted in very few rooted cuttings. Early survival counts indicated that cuttings from the lower part of the tree survived in greater percentages than those from the upper part and that lateral cuttings were superior to terminal in this respect (11). The percentage of rooted cuttings from early spring plantings in sand was negligible. Likewise, greenhouse propagation of dormant material taken throughout the winter has proven unsatisfactory; in several such experiments rooting was of the order of 10% and less.

Discussion

The results indicate that cuttings of white pine and white spruce can be rooted in substantial percentages under outside conditions. Period of collection and propagation medium appear to be the limiting factors. The optimum time of collection for white pine was mid- to late August, that for white spruce late July. Cuttings of both species taken before the optimum period showed low survival, which may be related to inadequate lignification. Collections of white pine taken later than the optimum period maintained a high percentage survival and it is possible that substantially higher rooting might have been obtained had the cuttings been left in the frames for a second season. In contrast to the white pine, white spruce cuttings taken after the optimum period demonstrated markedly poorer survival. The sedge type of peat medium gave the best results with both species; indeed it was an absolute necessity for any success with white spruce. White pine, on the other hand, did root to an appreciable extent in sand and sand mixed with sphagnum peat. The beneficial effect of sedge peat may be related to available nutrient factors (16). The results indicate that cuttings of these species root much better under outside conditions than in the greenhouse.

The data indicate that the latter half of August was the optimum period for collecting white pine cuttings. At that season the average rooting of cuttings propagated in sedge peat medium was about 60%. These cuttings were from trees 10 to 15 yr. of age. Cuttings of Experiments 3 and 4 were

from 16-yr. old trees which may account for the somewhat poorer rooting, since it is known that cuttings from older trees do not root so readily (30). Furthermore, it should be noted that these experiments were conducted at an unfavourable season, hence the relative responses of the various types of cuttings and media would not necessarily hold at the optimum season. This observation also relates to Experiments 7 and 8 with white spruce.

Rooting of white spruce cuttings was between 80 and 90% when taken late in July and planted in a sedge peat medium. While rooting of white spruce under optimum conditions approximates the high percentages readily achieved with Norway spruce, satisfactory results with the latter species may be obtained in collections taken over a much longer period. Further, the presence of a heel of old wood is generally favourable to white spruce but usually injurious with Norway spruce. Another striking difference relates to the superior development of new growth by Norway spruce cuttings (7, 8).

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THE EFFECTS OF AMOUNT AND DISTRIBUTION OF RAINFALL ON THE PROTEIN CONTENT OF WESTERN CANADIAN WHEAT[1]

By Allan E. Paull[2] and J. Ansel Anderson[3]

Abstract

Data for 14 yr. were used to determine the average effect of rainfall on the protein content of wheat grown at seven stations in the dry belt of southwestern Saskatchewan. Employing the concept of a regressional integral, and working with the rainfall for consecutive five-day intervals from April 1 to August 3, a curve was obtained which represents the change occurring during the growing season in the average regression coefficient (i.e., the average unit decrease in protein content per unit increase in rainfall). It was found that 34% of the residual variance for protein content can be ascribed to variations in rainfall. Above average rainfall during the growing season generally tends to reduce protein content, but this tendency is much more marked during April and early May, and during the latter part of July. These are also the periods during which average rainfall is lowest. The effect on protein content of precipitation occurring during the previous fall is of minor importance.

The study of the effects of weather on the yield and quality of cereal grains is as old as agricultural science itself and a voluminous literature on this subject has gradually accumulated (1, 5). Additional investigation has been stimulated in recent years by the development of advanced statistical methods which make possible a more objective, precise, and quantitative interpretation of the data. Although statistical studies have not yet achieved the goal of accurate prediction of yield and quality from meteorological data, a great deal has been learned of the nature and magnitude of the relationships involved. However, there is still need for additional information, and the present paper represents an attempt to obtain further quantitative data on the effects of variations in the amounts and distribution of seasonal rainfall on the protein content of wheat grown under dry-farming conditions.

Observational Data

The annual protein surveys of this laboratory have made available detailed data on the protein content of wheat grown in Western Canada during each of the past 14 yr. In the area covered by the survey there are a number of meteorological stations many of which have recorded daily precipitation data over the whole of this period.

From the two sets of data thus accumulated, it was necessary to select suitable series for the present investigation. As a first step, it was decided to limit the study to a comparatively small area, in one soil zone, in which

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gross differences in climate do not exist. The brown soils area in southwestern Saskatchewan was finally selected, not only because it met the above criteria, but also because it represented an area of low precipitation where variations from the average might be expected to have marked effects.

Examination of the Monthly Records of the Meteorological Service of Canada showed that it was possible to select, in the prescribed area, a total of six stations (Table I) having complete daily precipitation records for the

TABLE I

RAINFALL DISTRIBUTION COEFFICIENTS AND PROTEIN LEVELS FOR EACH STATION AND EACH YEAR

Station	Year	Rainfall distribution coefficients (in 0.01 in.)							Protein level, %
		ρ'_0	ρ'_1	ρ'_2	ρ'_3	ρ'_4	ρ'_5	z	P
Swift Current	1927	49.28	−1.02	−6.90	0.91	0.08	−3.70	525	11.1
	1928	34.92	11.32	−2.23	−3.35	2.27	3.90	580	13.1
	1929	29.36	2.80	−5.96	−3.88	1.54	0.13	112	15.1
	1930	27.92	1.05	−5.15	−1.97	−1.39	2.18	221	15.2
	1931	19.60	6.80	−0.53	−1.74	−0.49	1.31	528	15.1
	1932	43.44	11.67	−2.00	−3.55	−1.93	1.39	467	14.6
	1933	27.24	−0.38	−4.48	−2.33	0.57	0.08	480	15.2
	1934	28.84	7.63	−5.24	−2.56	1.71	0.36	739	15.2
	1935	40.48	9.26	−0.21	−0.08	−0.72	2.09	263	14.7
	1936	19.96	0.95	−4.39	−0.57	−0.35	0.32	276	15.4
	1937	12.12	3.49	0.46	0.47	1.23	−0.20	290	16.2
	1938	21.08	−0.37	−4.23	1.07	0.31	0.06	250	14.0
	1939	45.88	5.80	−9.16	−2.33	3.67	0.20	407	13.2
	1940	28.28	2.15	1.11	−1.77	5.09	−0.01	155	12.8
Regina	1927	42.76	8.20	−1.99	−4.93	−1.02	−2.04	413	11.9
	1928	38.56	5.50	−7.13	−5.17	2.15	3.12	689	13.0
	1929	17.32	−0.98	−3.67	−1.41	1.94	0.31	107	13.9
	1930	21.84	0.12	−3.51	−0.19	−0.03	0.63	217	14.0
	1931	20.32	9.16	−0.45	−3.11	−1.84	0.12	285	14.8
	1932	28.20	3.00	−5.95	−2.02	0.80	1.52	380	15.6
	1933	35.20	−2.51	−7.99	−0.87	4.21	−2.57	380	14.7
	1934	22.48	5.59	−2.51	−1.36	0.97	−0.26	467	14.7
	1935	45.36	5.83	−5.03	2.72	3.88	2.04	335	13.9
	1936	25.36	−0.26	−6.31	−0.21	0.09	0.75	434	15.0
	1937	19.28	4.54	−1.83	−0.05	1.29	−0.10	224	15.6
	1938	25.92	2.51	−3.79	−0.77	−1.50	0.18	252	14.9
	1939	32.64	7.54	−7.16	−5.76	0.80	2.37	265	15.5
	1940	22.44	3.94	0.96	−1.49	2.24	−0.58	253	15.1
Moose Jaw	1927	31.08	1.35	−4.86	0.51	0.65	−2.37	526	11.5
	1928	36.36	5.70	−8.26	−6.07	2.13	2.82	355	12.9
	1929	14.16	−0.40	−3.59	−0.64	1.22	−0.08	162	15.2
	1930	21.56	1.27	−1.20	0.43	−0.52	0.33	228	15.0
	1931	21.96	7.66	−3.18	−3.40	0.21	1.98	385	14.6
	1932	39.80	5.92	−9.42	−4.75	1.37	2.24	531	14.5
	1933	37.92	−2.13	−8.91	−0.74	3.10	−0.96	451	14.9
	1934	23.12	4.86	−4.79	−1.70	1.74	0.26	533	14.7
	1935	44.64	5.74	−0.71	6.63	1.19	0.09	286	12.8
	1936	30.04	−1.45	−6.43	2.18	−0.76	−0.97	412	14.6
	1937	19.96	6.47	1.99	−0.21	−0.26	−2.36	145	14.9
	1938	26.96	0.49	−3.91	−0.22	−2.24	0.72	231	13.9
	1939	32.36	3.46	−9.15	−3.11	3.34	2.30	279	14.2
	1940	29.24	4.03	0.13	−1.73	3.88	−0.86	136	14.4

TABLE I—*Concluded*

RAINFALL DISTRIBUTION COEFFICIENTS AND PROTEIN LEVELS FOR EACH STATION AND EACH YEAR
—*Concluded*

Station	Year	Rainfall distribution coefficients (in 0.01 in.)							Protein level, %
		ρ'_0	ρ'_1	ρ'_2	ρ'_3	ρ'_4	ρ'_5	z	P
Beechy	1927	37.44	1.46	−2.94	0.08	−0.50	−1.91	325	12.0
	1928	28.16	3.71	−2.35	−3.25	1.24	2.46	629	12.7
	1929	19.76	−0.76	−4.32	−1.56	0.92	1.26	172	15.0
	1930	23.80	3.94	−2.17	−1.05	−1.39	−0.40	207	15.6
	1931	17.52	6.70	0.93	0.06	0.40	2.52	433	15.3
	1932	28.60	6.33	−2.28	−3.00	−0.92	0.86	444	15.3
	1933	19.28	−1.49	−2.71	−0.76	1.15	−1.06	366	15.7
	1934	17.64	2.91	−5.46	−1.63	2.45	0.59	486	16.4
	1935	40.68	8.68	−2.12	−2.32	−3.03	0.87	159	15.6
	1936	24.16	2.96	−4.18	−0.09	−0.51	−0.97	328	15.9
	1937	28.24	7.94	0.58	0.74	0.09	−2.51	267	15.9
	1938	20.60	2.01	−2.62	0.79	−0.39	0.06	205	15.4
	1939	43.80	9.47	−10.48	−5.67	2.36	1.83	395	14.3
	1940	24.00	6.21	1.04	−0.56	1.46	−0.54	136	15.0
Assiniboia	1927	22.36	0.73	−4.46	0.49	0.70	−1.33	507	12.8
	1928	38.28	8.71	−4.07	−7.12	1.02	2.12	252	13.3
	1929	6.76	−0.06	−1.48	−0.47	0.32	0.00	132	15.8
	1930	20.52	−0.44	−2.45	−0.31	−0.23	0.65	235	16.4
	1931	24.60	10.66	0.27	−1.56	0.78	2.23	271	15.6
	1932	29.44	2.75	−7.92	−3.41	1.60	2.50	416	16.6
	1933	30.68	−0.73	−6.56	−1.68	1.35	−0.32	363	16.0
	1934	22.92	3.48	−5.71	−1.17	1.91	−0.01	631	15.6
	1935	48.16	5.79	−7.26	−1.21	0.49	1.23	225	15.3
	1936	16.84	2.56	−1.28	0.65	−0.95	−0.34	289	16.5
	1937	19.56	8.33	3.25	−1.50	−0.35	−0.92	211	16.4
	1938	41.28	4.61	−6.55	−2.16	−3.04	0.51	403	14.7
	1939	46.24	3.43	−12.70	−3.55	3.51	1.67	381	14.4
	1940	41.40	5.44	−0.07	0.00	4.78	−0.48	153	13.7
Anglia	1927	42.84	1.40	−5.45	1.24	−1.31	−2.54	195	11.6
	1928	32.28	9.75	−0.81	−1.75	1.41	2.21	468	13.4
	1929	29.68	1.24	−3.86	−4.99	0.96	1.27	314	13.7
	1930	25.60	5.34	2.20	−0.47	−2.24	−0.05	160	13.7
	1931	11.96	4.28	0.35	−2.12	−1.22	0.64	437	14.4
	1932	17.36	3.33	−1.78	−2.01	−1.42	0.17	285	14.4
	1933	11.28	−0.26	−1.55	−0.22	−0.35	−0.57	226	14.8
	1934	12.60	3.05	−2.59	−2.84	−0.14	0.99	318	15.7
	1935	24.92	2.36	0.56	−1.84	−1.23	1.58	99	14.8
	1936	10.40	−1.06	−1.51	−0.31	−0.19	0.74	185	15.6
	1937	18.40	6.15	−0.33	−1.94	−1.05	−1.45	146	15.1
	1938	29.44	0.78	−2.30	1.64	−0.04	1.33	220	13.9
	1939	30.36	3.94	−8.95	−4.89	2.67	2.68	477	14.3
	1940	35.40	7.33	−0.41	−4.12	−0.24	−0.42	184	13.7
Alsask	1927	62.72	3.98	−5.02	−4.10	−3.12	−2.04	398	11.0
	1928	30.48	2.26	0.32	−2.86	1.39	0.92	386	13.4
	1929	19.84	−1.59	0.54	−3.03	1.08	−2.31	288	15.2
	1930	40.36	6.63	−0.95	−0.12	−1.88	3.50	108	14.2
	1931	13.88	4.26	0.19	−1.79	−1.56	−0.16	602	14.7
	1932	37.60	−0.52	−2.09	−3.04	0.64	−1.34	288	14.5
	1933	14.44	0.21	−1.85	−0.26	−0.99	−0.54	347	15.0
	1934	21.48	0.78	−4.73	0.19	0.59	0.52	393	15.9
	1935	21.48	−0.38	−0.99	−0.61	−0.57	1.10	205	16.3
	1936	18.96	1.64	−2.61	−0.35	−0.79	0.01	291	16.8
	1937	16.68	4.34	−0.41	−1.03	0.22	−0.59	316	15.5
	1938	32.56	5.70	−1.64	−0.76	−1.54	1.46	218	15.2
	1939	32.56	2.83	−8.11	−2.90	2.54	−0.32	303	15.4

14 yr. period. A seventh station, Alsask, was also added, though daily records were not complete for 1940.

The protein level for wheat grown around each meteorological station was taken as the mean of results for all samples taken from carlots shipped from points within a radius of 15 miles of the station. In so far as precipitation records taken at a central point may be considered representative of such an area, this procedure seemed desirable since it tends to reduce sampling error and thus to produce a more reliable series of values. The annual protein levels for each station are given in the last column of Table I. Each figure represents the mean of results obtained for an average of 19 samples: the lowest number of samples represented is one, and the highest is 40.

The study of the effects on protein content of amount and distribution of precipitation was confined to the period represented by the months of April, May, June, July, and the first three days of August. For purposes of calculation this period was divided into 25 intervals of five days each.

Account was also taken of the precipitation occurring during the three months, August, September, and October, of the fall preceding the growing season under study. However, no attempt was made to study the effect of distribution of rainfall during this period, the total amount of rainfall for the three months was treated simply as a single additional rainfall variant.

The practice of considering the precipitation affecting the crop as that occurring during the growing season and during three months of the previous fall, and of disregarding winter precipitation, is now generally adopted in Western Canada. The practice stems from the investigations of Barnes and Hopkins (2) who state, in a study of crop production at Swift Current, that "no appreciable increase in soil moisture has been observed from the presence of snow." In Western Canada precipitation during the winter months occurs as snow, which falls on frozen ground and cannot be absorbed. An appreciable proportion of the snow is lost by evaporation and most of the remainder is lost in the spring run-off because the snow melts before the underlying ground can thaw.

Methods of Analysis

Fisher's concept (4) of a regression integral, involving a regression function varying continuously with time, was employed in this investigation. Details of the practical application of this concept to the study of the effect of rainfall on the yield of Rothamsted plots are given in full by Fisher (4), together with a thorough discussion of the advantages and disadvantages of the method. Hopkins (7) also employed this method in his study of the effect of rainfall on the yield of wheat at certain stations in Western Canada. He worked with data for eight stations, 10 years, and a 125 day period divided into 25 five-day intervals, thus providing a pattern for the present study. In these circumstances the method need not be fully described in this paper.

In this study, protein content at any station is taken as a function of the amount and distribution of rainfall over the growing season and the amount of rainfall for the previous fall. The regression integral therefore takes the form

$$P = c + \int_0^T a(t)r(t)dt + kz$$

where $a(t)$ represents the effect on protein content of a unit increment of rain falling at time t, and $r(t)dt$ the increment of rain falling in the time interval dt; the integral is taken over the 125 days of the growing season. The last term takes account of the previous fall rain, z representing the amount and k its regression coefficient. The quantities to be evaluated from the available data are $a(t)$ and k.

Following Fisher's method, the seasonal rainfall, $r(t)$, was expressed as a function of time in terms of orthogonal polynomials of the fifth degree, there being one equation for each station in each year. Each equation is fitted (13, pp. 279-289) to the appropriate set of 25 data representing rainfall for five-day intervals during the growing season. The six coefficients, ρ'_0 to ρ'_5 (corresponding to Fisher's coefficients a' to f'), of each equation were then available as independent rainfall variables with which to correlate the protein data, P. As pointed out previously, there was also available a seventh set of rainfall variables, z, representing the precipitation of the previous fall. Values of ρ', z, and P, for each station in each year, are listed in Table I.

The correlations were based on the residuals after removing the variation due to stations (six degrees of freedom) and years (13 degrees of freedom). This procedure is adopted for several reasons. Although the seven stations selected are in a limited area, it is not valid to assume that the only factors affecting protein content are seasonal variations in rainfall and factors closely associated with it. The annual protein values for each station show differences from year to year which are not ascribable to changes in rainfall. These might arise from differences in dates of seeding, or in the soils, percentages of summer-fallow, etc., represented by the protein samples. Again, in the area represented one might expect a certain correlation to exist between the weather at different stations, and if some extraneous source of variation is common to several stations, a spurious effect will be introduced into the regression. Such interannual heterogeneity is partially eliminated by working with residuals which should represent a homogeneous group of data whose variations arise primarily from the variations that exist within the particular year from which they are drawn. Correlations of these data thus serve to illustrate the effect on the average protein content of the wheat of deviations from the average amount and average distribution of the rainfall.

Fifth degree polynomial functions were fitted to the ρ', z, and P values for each station giving rise to sets of six coefficients, x_0 to x_5, describing the interannual trends in the data. The values for x, together with the calculated

standard residues, are listed in Table II. Fisher (4), working with continuous series of 60 yr. used x values such as these for eliminating the heterogeneous variation occurring between years. However, in the present investigation, since the values from several stations in a relatively small area are to be posted, it was thought best to remove the interannual variations by adjusting the annual averages over the seven stations. The x coefficients were used simply to examine the interannual tendencies of rainfall and protein over the 14 yr.

TABLE II

ANALYSIS OF INTERANNUAL TRENDS OF RAINFALL AND PROTEIN

Station		ρ'_0	ρ'_1	ρ'_2	ρ'_3	ρ'_4	ρ'_5	z	P†
Swift Current	Mean	30.600	4.368	−3.494	−1.549	0.828	0.579	378.1	43.500
	x_1	−11.372	−1.970	2.256	1.791	3.092	−0.417	−218.8	7.326
	x_2	13.372	−4.186	−1.785	0.835	4.348	−2.192	−127.2	−40.991
	x_3	− 2.122	2.847	2.149	−4.092	1.394	2.454	−126.0	5.888
	x_4	14.519	0.287	1.899	0.262	0.387	−2.190	253.8	−16.392
	x_5	− 9.662	2.661	4.222	−1.046	1.966	2.357	−177.2	9.358
	Standard residue	10.849	5.153	3.236	1.298	1.336	1.412	177.3	5.838
Regina	Mean	28.406	3.727	−4.026	−1.759	0.999	0.392	335.8	44.714
	x_1	− 6.824	−0.036	0.916	2.426	0.711	0.380	−155.6	28.244
	x_2	5.392	4.147	2.854	−4.978	−1.133	−0.087	− 7.6	−15.492
	x_3	−14.786	−2.371	2.015	−0.487	0.760	0.419	−163.6	11.455
	x_4	12.522	2.370	1.873	0.216	1.762	−2.720	165.3	− 5.746
	x_5	− 1.196	−3.590	0.285	3.264	3.568	1.335	117.3	− 6.512
	Standard residue	8.725	3.976	3.119	1.869	1.753	1.675	145.7	4.854
Moose Jaw	Mean	29.226	3.069	−4.449	−0.916	1.075	0.224	332.9	41.500
	x_1	2.526	0.514	2.935	2.116	0.638	−0.587	−211.6	11.370
	x_2	− 2.780	−0.584	1.061	−2.588	1.587	−0.614	−166.5	−18.198
	x_3	− 5.554	1.603	0.124	−4.882	2.613	2.617	−131.9	20.630
	x_4	14.426	0.783	−1.115	1.346	3.047	−1.886	290.1	−11.868
	x_5	− 0.645	−0.088	4.269	2.930	0.943	−1.065	−164.7	1.689
	Standard residue	9.376	3.995	4.270	3.154	1.520	1.722	92.6	7.087
Beechy	Mean	26.691	4.291	−2.791	−1.301	0.238	0.219	325.1	50.071
	x_1	3.440	5.662	−1.026	−0.028	0.888	−1.010	−184.8	22.177
	x_2	10.762	0.222	−0.474	−0.485	1.510	−0.351	− 69.5	−33.838
	x_3	−11.677	0.834	2.048	−1.472	1.381	2.457	− 36.8	9.521
	x_4	2.548	−0.549	1.792	0.422	0.138	−1.488	37.4	− 3.277
	x_5	− 4.654	−1.168	2.248	0.261	−0.143	0.059	− 71.5	5.244
	Standard residue	9.001	3.789	3.500	2.208	1.746	1.643	166.7	5.306
Assiniboia	Mean	29.217	3.947	−4.071	−1.643	0.849	0.536	319.2	52.214
	x_1	21.502	2.632	−1.608	0.912	1.257	−0.552	− 37.4	4.674
	x_2	9.534	0.394	0.576	−0.381	2.180	−1.051	− 76.0	−38.100
	x_3	0.947	−0.280	1.256	−0.552	3.430	1.791	−192.7	7.203
	x_4	7.372	−1.940	−1.730	2.218	3.613	−1.134	201.8	−10.348
	x_5	− 4.494	1.768	6.190	0.225	0.392	−0.883	−248.1	− 0.396
	Standard residue	12.906	4.335	4.669	2.421	1.351	1.277	115.2	7.104

† *Protein coded: $P = 10$ (percentage of protein -10).*

TABLE II—*Concluded*

ANALYSIS OF INTERANNUAL TRENDS OF RAINFALL AND PROTEIN—*Concluded*

Station		ρ'_0	ρ'_1	ρ'_2	ρ'_3	ρ'_4	ρ'_5	z	P†
Anglia	Mean	23.751	3.402	−1.888	−1.759	−0.314	0.470	265.3	42.214
	x_1	− 5.112	−0.384	−1.028	−1.588	1.095	0.986	− 90.0	18.862
	x_2	32.876	4.156	−4.192	−0.824	1.718	−0.688	68.5	−28.909
	x_3	− 2.417	3.938	2.633	−3.896	0.002	0.963	129.2	− 0.083
	x_4	− 1.450	−1.386	0.124	0.601	−1.507	−2.351	−114.8	− 1.740
	x_5	0.173	2.750	4.092	−2.014	0.549	0.990	− 98.4	8.736
	Standard residue	5.483	3.078	2.705	1.971	1.371	1.458	133.8	4.116
Alsask	Mean	27.926	2.138	−2.104	−1.589	−0.307	0.016	318.7	48.538
	x_1	−18.407	0.821	−3.122	2.268	1.562	1.024	−106.6	36.321
	x_2	27.764	3.496	−3.638	−3.015	0.463	−0.900	− 11.2	−25.344
	x_3	− 5.449	0.793	0.159	−0.758	2.173	0.847	− 13.3	3.763
	x_4	15.124	−1.858	−6.262	−1.033	−0.215	−1.492	121.2	−13.362
	x_5	−13.163	−5.023	−1.118	0.176	3.476	0.262	− 41.4	16.552
	Standard residue	10.010	2.286	1.315	1.105	1.173	1.848	141.3	4.158

† *Protein coded:* $P = 10$ *(Percentage of protein* $- 10$*).*

Finally, the regression function $a(t)$ was expressed in terms of orthogonal polynomials of the fifth degree involving the coefficients α_0 to α_5. The equation then takes the form

$$P = c + \alpha_0\rho_0 + \alpha_1\rho_1 + \alpha_2\rho_2 + \alpha_3\rho_3 + \alpha_4\rho_4 + \alpha_5\rho_5 + kz$$

The actual calculations were made with the residuals of ρ' and z, obtaining, by the method of least squares, a set of coefficients α' from which the values of α were derived. These statistics, α values and k, are recorded and discussed in a later section. The function thus evaluated makes it possible to determine the effect on protein content of an additional increment of rainfall occurring at any period during the growing season, or during the three month period of the previous fall.

The method of analysis, based on the concept of a regression integral, has certain advantages over the more commonly used multiple regression method. To divide the season into 25 five-day intervals and obtain the regression on each would entail the solution of determinants of order 26, an undertaking involving an enormous amount of calculation. Moreover, the loss of 26 degrees of freedom would decrease considerably the precision of the test of significance. It should also be noted that the multiple regression method makes no use of the well founded assumption that the regression coefficient of protein content on rainfall is a slow changing function of time.

Two possible objections to the use of the regression integral are worth noting. In the first place, rainfall is not a continuous function of time over the growing season and yet it is so treated. Although there is no reason to believe that this treatment will introduce a bias, it does tend to decrease efficiency by introducing an additional source of variation. Fisher disposes

of the point by suggesting that since the regression function "varies relatively slowly, little would be gained by following, in more detail, the rapid fluctuations of the weather." The second objection relates to the assumption that the effect of rain falling at any time is independent of rain falling previously in the same growing season. Although such a premise may be open to question, it appears reasonably valid for a region in which the soil is seldom, if ever, saturated.

Analysis of Rainfall

The average amount and distribution of seasonal rainfall, for all stations over the 14 yr., are illustrated in Fig. 1. In drawing the graph the average amount of rain falling in each five day interval was plotted against the middle date for the interval. The curve shows that rainfall is normally low in April, rises steadily during May, reaches a peak in June, and decreases during July. This trend is similar to that obtained by Hopkins (9) with rainfall data for 48 yr. at Swift Current, one of the stations included in the present study.

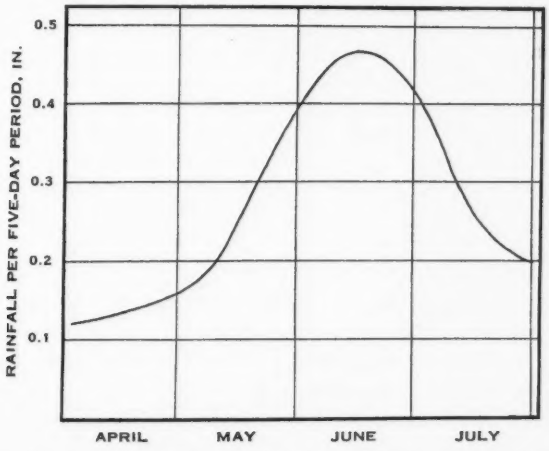

FIG. 1. *Average precipitation recorded at seven stations in southwestern Saskatchewan, 1927–1940.*

The total average rainfall for the period April 1 to August 3 is seven inches, while the greatest average amount falling in any five day interval is 0.46 in. Such moisture conditions are very close to the minimum necessary for the growth of wheat. If variations in rainfall do affect the protein content of the crop, one would expect to observe a magnification of this effect under such dry conditions.

The characteristics of the seasonal rainfalls at each station are represented by the ρ' coefficients of Table I. There is one line of data for each year at each station. The first column of data, under ρ'_0, presents the average value of the five-day totals of rainfall for the season. Data in the next column,

ρ'_1, measure the linear or average rate of increase of this rainfall; ρ'_2 measures the quadratic term in the sequence of rainfall totals; ρ'_3, the cubic term, and so on. Since these statistics represent only the raw data on which this study was based, no attempt will be made to discuss them in detail.

The results of the analyses of the interannual tendencies of the rainfall data for each of the stations are given in Table II. The x coefficients are similar in nature to the ρ' coefficients described above: x_1 measures the linear trend, x_2 the quadratic trend, and so on. The standard residue is taken as $\left(\sum_6^{13} x\right)/8$, assuming that all slow changes have been accounted for by x_1 to x_5.

In dealing with a short period of 14 yr., one would not expect an analysis of the data to produce conclusive evidence of progressive changes in the amount and distribution of rainfall from year to year. In general, this expectation was borne out in the present study. However, examination of Table II will show that at Alsask and Anglia the magnitude of the quadratic terms (x_2) in the ρ'_0 sequence indicates that a series of dry years occurred between 1931 and 1937. These together with significant x_1 and x_2 values in the Alsask distribution coefficients ρ'_2 and ρ'_3, suggest the presence of progressive change. It is well known, of course, that a cycle of drought years did occur in southern Saskatchewan between 1931 and 1937, and it seems probable that the significance of this trend could be established by the study of data for a long period of years. However, in the present study, which deals with only 14 yr., interannual differences in rainfall must be considered to arise fortuitously, and it must be concluded that the climatic conditions of the area did not exhibit any marked changes.

Regression of Protein Content on Rainfall

The following values were obtained for the coefficients of the equation representing the regression of protein content on rainfall (see p. 218).

$$\alpha_0 = -0.1213 \quad \alpha_1 = 0.0713 \quad \alpha_2 = -0.0654 \quad k = -0.00811$$
$$\alpha_3 = -0.0497 \quad \alpha_4 = 0.00881 \quad \alpha_5 = 0.00001$$

Protein was measured in units of 0.1% and rainfall in 0.01 in.

The significance of the correlation between protein and the various rainfall coefficients may be determined by analysing the variance of the protein residuals into portions due to the rainfall regression and to deviations from the regression. The results of the analyses are shown in Table III. From the first analysis it is apparent that the correlation coefficient ($R = .632$) is highly significant, the 1% point being .475. Following Fisher's procedure (4), the percentage of the residual variance accounted for by variations in rainfall was calculated and found to be 34%, a high proportion for a single meteorological variant. The remaining 66% is accounted for by environmental factors not associated with rainfall, and by fortuitous experimental errors. These latter may arise from various causes, among which may be mentioned:— differences in seeding times; differences in the soils represented at each station in each year; and differences in the effectiveness of equal

increments of rain falling at each station in each year, it being assumed that a number of light showers are less effective than a steady rain.

More detailed analyses are also presented in Table III. Analysis 2 shows that the major portion of the variation is accounted for by variations in the total seasonal rainfall (ρ_0) although the remaining rainfall variables still have a variance significantly greater than the error. Statistics given in Analysis 3 show that the introduction of fall rainfall does not reduce the variance due to the remaining variables, suggesting that ρ_1, ρ_2, and possibly ρ_3 have a greater effect on the protein than z. Analysis 4 illustrates the additional variation accounted for when ρ_1, ρ_2, and ρ_3 are introduced, and shows that ρ_4, ρ_5, and z are unimportant in the present investigation. It thus appears that a satisfactory correlation can be obtained by considering only the amount of seasonal rainfall along with its distribution as expressed by a cubic function of time.

TABLE III

ANALYSIS OF VARIANCE OF PROTEIN RESIDUALS

Analysis number	Variance due to	Degrees of freedom	Sum of squares	Mean square
1	Rainfall regression $R = .632$	7	1178	168.3**
	Deviations from regression	70	1776	25.4
2	Total seasonal rainfall	1	743.6	743.6**
	Remaining rainfall variants	6	434.4	72.4*
	Deviations from regression	70	1776	25.4
3	Total seasonal rainfall	1	743.6	743.6**
	Fall rainfall	1	31.6	31.6
	Remaining rainfall variants	5	402.8	80.6*
	Deviations from regression	70	1776	25.4
4	Total seasonal rainfall	1	743.6	743.6**
	Linear trend	1	79.6	79.6
	Quadratic trend	1	199.1	199.1**
	Cubic trend	1	113.4	113.4*
	Remaining rainfall variants	3	42.3	14.1
	Deviations from regression	70	1776	25.4

* *The variance surpasses the 5% level of significance.*
** *The variance surpasses the 1% level of significance.*

The regression coefficients, given in the first paragraph of this section, make it possible to estimate the average effect on the protein content of the crop of an additional inch of rainfall at any time during the growing season. The difference in the effect of additional rain falling at different periods, i.e., the course of the regression function $a(t)$, is shown by the curve in Fig. 2.

Before considering the curve in detail, it seems best to dispose of the effect of an increase in the total rainfall for the growing season. Returning to the regression coefficients, it will be observed that the comparative magnitude of α_0 indicates that total precipitation has a major effect on protein content.

Decoding the value for α_0, and expressing the result in simple terms, it may be stated that if the total rainfall is increased one inch above normal, while the rainfall distribution is held constant, protein content tends to decrease by about 0.24%.

The curve in Fig. 2 indicates that additional rainfall generally tends to decrease the percentage of protein in the wheat. It is also clear that the effect of a given increment of additional rainfall depends upon the period during which it falls. In this matter the curve speaks for itself, but summarizing, it may be said that additional rainfall occurring in April, the first half of May, or the last half of July, has an appreciably greater effect on protein content than rain falling during June.

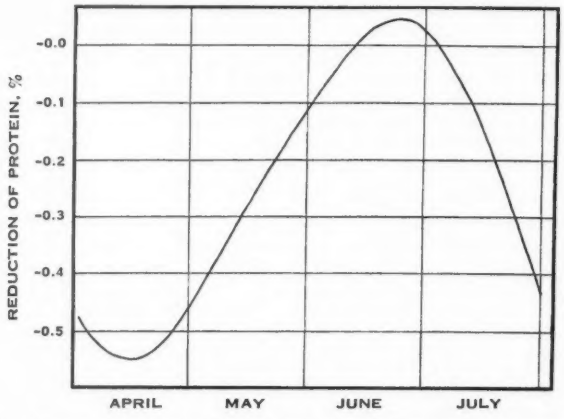

FIG. 2. *Average effect of an additional inch of rainfall on the protein content of wheat.*

When interpreting the curve, the reader is warned not to generalize too broadly. The curve deals with the average amount and distribution of seasonal rainfall, and the average annual protein level of wheat, at seven stations in the dry area of southwestern Saskatchewan. It illustrates the effect of deviations from the average rainfall in terms of the resulting deviations of the percentage protein from its average value. The curve does not show that each inch of rainfall in early May, for instance, will lower protein content by 0.4%, but only that an increase of an inch over the normal rainfall will result in an average decrease of 0.4% in the normal protein level. The results are actually reported in terms of the effect of an additional inch of rain on percentage protein merely because this is the customary practice. It would be equally correct to report the results in terms of the effect of one inch less rainfall than normal, and perhaps better to report in terms of the effect of a difference in rainfall of from one-half inch below normal to one-half inch above normal. For that matter, it is not necessary, and perhaps even slightly misleading, to select the conventional one inch of rainfall as the reference unit.

However, it will be clear that no change in the regression is involved if it is reported in terms of the effects of one-tenth rather than one inch of additional rainfall.

A further difficulty may arise from the fact that a continuous, smooth curve suggests that the interpretation should be limited to the effect of an additional increment of rain falling at a given time during a very short period. Actually, a consideration of the data on which the curve is based suggests that a detailed interpretation of the curve is not warranted. The additional increment of rain may better be thought of as occurring during a longer period, e.g., the first half of June, when its effects will be represented by the average level of the curve for this period. This procedure seems much sounder, particularly in view of the fact that in the area concerned the average amount of rain falling in June is less than 3 in., and June has normally a higher rainfall than any other month of the growing season.

Returning to the coefficients listed on page 220, it will be noted that the coefficient (k) of the partial regression of protein content on rainfall occurring in the three months of the fall preceding the growing season is -0.0081. In other words, an inch more rain than normal in the fall decreases the average protein content of the next crop by only 0.08%.

Discussion

Previous statistical investigations of the effect of rainfall on protein content, under semiarid conditions approximating those of the present study, appear to be confined to those of Hopkins (6, 8). He worked with data, collected in certain years during the period 1915 to 1932, for five stations in Western Canada (Lacombe, Lethbridge, Indian Head, Scott, and Rosthern). The average conditions represented in Hopkins' study are similar to those of the present study, as can be seen by comparing the mean total rainfalls for the period May 1 to July 31, which are: Hopkins, 6.4 in.; this study, 6.2 in. It should be noted, however, that there is probably a difference in the average dates of seeding and heading of wheat, those for the present study being somewhat earlier.

Working with the partial regressions of protein content on monthly rainfall totals for May, June, July, and August, Hopkins (6) found that the main effect of rainfall in reducing protein content was exerted during May and June. His data did not justify the conclusion that the amount of rain falling in July or August, or the amount of preseasonal precipitation (August 1 to April 30), modified the nitrogen content significantly. Mean maximum temperature for July or August also failed to show a significant correlation with nitrogen content. However, in a second paper (8), by the use of coefficients designed to weight observed temperatures in proportion to their assumed effect on the respiration of the grain, higher temperatures during the six weeks preceding September 1 were shown to have a moderate positive correlation with protein content.

The results of the present investigation support Hopkins' conclusions concerning the importance of additional rain falling during the early part of the growing season. However, whereas Hopkins found that May rainfall had the greatest effect on protein content, followed closely by June rainfall, in the present study the April rainfall is most effective, May rainfall is moderately effective, and June rainfall is only of minor importance. This difference may be accounted for in part by some difference in the average dates of seeding and of heading represented in the two investigations. Unfortunately, it was not possible to obtain complete data on this point but it seems safe to assume that the average dates of seeding and heading are slightly later for the group of stations examined by Hopkins. It should also be noted that Hopkins did not examine April rainfall which might also have been more effective than May rainfall under the conditions that he studied.

Russell and Bishop (11) in their investigation of the effect of weather on the nitrogen content of barley, also found that rainfall occurring early in the growing season had a major effect on the nitrogen content of the kernels. They suggest that excessive spring rain may reduce nitrogen content by leaching nitrates from the soil. This hypothesis, as Hopkins points out, can hardly apply under the semiarid conditions of the southern and south-central portions of the Prairie Provinces. Under such conditions, Hopkins believes that the main effect is produced in another way, namely, by the stimulation of tillering and general vegetative development produced by early rains. He writes, "as a result of this proliferation, the supply of available nitrogen must be distributed amongst an increased number of culms, and the total leaf area, devoted to the production of carbohydrates, will also be augmented, both circumstances tending towards a diminished proportion of nitrogen in the resulting grain."

This hypothesis appears to require some extension to account for the results illustrated in Fig. 2. Specifically, it seems necessary to provide an explanation for the fact that under dry conditions additional rain occurring in April, prior to seeding, depresses protein content appreciably more than rain occurring when the wheat starts to tiller. On the other hand, it would be unwise to pay too much attention to shape of the curve for April, and particularly to the exact position of the minimum point, since the available data do not serve to establish these with certainty.

The average date of seeding, under the conditions represented in this study, is estimated to be April 30. Fig. 2 shows that additional rainfall has its maximum effect about April 15. It thus seems probable that the mechanism involved at this time is concerned with the condition of the seed bed, and hence with the germination and early growth of the seeds. With rainfall normally very low in April, it appears that above average precipitation may result in an appreciably higher germination than normal and in a heavier stand of more vigorous seedlings. If an average summer then follows, the good start obtained by the crop will result in above normal vegetative growth and thus in a decrease in the protein content of the crop.

It will be observed that the curve in Fig. 2 has a minimum value at about the middle of April, i.e., about two weeks before seeding. Additional rain falling prior to this time appears to have less effect on the protein content of the crop, as does the precipitation occurring in the three months of the preceding fall. Assuming this hypothesis to be correct, some explanation of it may be attempted. On summer fallowed land, such rain will fall on bare ground and may be expected to be less available to the seed for two reasons. In the first place it will gradually penetrate below seeding depth, and in the second place it may be expected to evaporate more rapidly than it would do were the land partially covered as it is as soon as the wheat plants emerge. The first of these reasons applies equally well to stubble land and the second also applies in part if the stubble is worked before seeding. In consequence it does not seem improbable that precipitation occurring during the previous fall and some time before seeding should have less effect on germination and early vegetative growth than rainfall occurring just before seeding time.

In general it appears that in southwestern Saskatchewan rain is available to and used by the growing plants immediately after it falls, but its effect must continue for some time, thus carrying the plants over from one shower to the next. At the beginning of the growing season the young plant, with its short roots, is almost wholly dependent on recent rainfall. As growth continues, and the roots penetrate to greater depths, the plant is able to draw on additional reserves of moisture which have accumulated as a result of earlier rains. Accordingly, it would appear that tillering, which starts early in June, is affected not only by the rain falling during late May, but also by the amount of reserve moisture in the soil. Hence it seems reasonable to assume that rain falling in April may have a fairly direct effect on the vegetative growth occurring more than a month later. It thus appears that the hypothesis put forward by Hopkins (6), concerning the effect of additional rain early in the season on tillering and on the general vegetative growth of the plant, presents a reasonable explanation of the available data.

The curve in Fig. 2 shows that the protein content of the crop is very susceptible to variations from the normal rainfall during the latter part of July. This supports the work of Shutt and Hamilton (12), who concluded, as a result of studies on wheat grown at Scott, Saskatchewan, and at three stations in relatively high rainfall areas, that the "chief environmental factor conducive to high protein content" is "the drying out of soil, due to scanty precipitation and high temperatures, during the latter weeks of development and ripening of the kernel." Many other authors have expressed similar opinions. In his first paper, Hopkins (6) was unable to demonstrate that differences in either rainfall or temperature during the later part of the growing season had any marked effect on protein content. However, as noted above, he did show in his second paper that high temperatures had a moderate effect on protein content. Citing the chemical investigations of McCalla and Newton (10) and of other earlier workers, Hopkins assumes that the effect of high temperature is accounted for principally by increased respiration in

the grain and that it is not necessary to suppose that high temperatures change the rates at which carbohydrates and proteins are translocated to the kernels.

It seems probable that under the dry conditions represented in the present study, deviations from the normal rainfall at the end of July have an effect on the protein content of the grain which is not necessarily associated with any change in air temperature. Under normal conditions, wheat plants grown in southwestern Saskatchewan dry out fairly rapidly during the latter part of the growing season. If the rainfall is below normal at this time, the moisture content of the soil becomes a limiting factor, and it may be assumed that the rate of translocation of both proteins and carbohydrates diminishes rapidly and may even approach zero. If translocation decreases considerably, the effect of respiration in the grain on the subsequent protein content will obviously be of major importance. This mechanism will operate irrespective of air temperatures though it will be of increased importance with higher temperatures. Though it applies only to dry conditions, this hypothesis serves to explain how rainfall in late July may exert an effect on the protein content of the grain which is not directly associated with the possible effects of temperature. On the other hand, since some correlations may be expected between temperature and rainfall, it seems probable that the slope of the curve in Fig. 2 for July represents in part a temperature effect. There is some reason to suppose, however, that the curve for this period represents principally the effect of additional rainfall which maintains the rate, and increases the time, of translocation, and thus offsets the effect of respiration on protein content.

Superficially it might appear that the hypothesis outlined above is not consistent with results of studies made by Hopkins (7) and by Davis and Pallesen (3) of the effects of rainfall on yield. The former in his study of Marquis wheat, and the latter in their study of spring wheat, found that additional rainfall occurring more than 90 days after seeding tends to reduce the yield of wheat. In his summer fallow and stubble series, Hopkins found no such detrimental effect although the benefits of late rains were much less than those resulting from additional rainfall earlier in the season. The author suggests that the detrimental effect illustrated in the Marquis study may result from lodging, a factor that would not influence protein content. It seems reasonable to assume that Hopkins, like the present authors, is unable to explain diminished yield with increased late rainfall in terms of a differential effect of increased rainfall on rates of translocation and respiration.

One other feature of the data deserves close attention. It will be observed that there is considerable similarity between the average rainfall curve of Fig. 1 and the regression curve of Fig. 2. Additional rainfall obviously has its greatest effect at periods when the average rainfall is low. This suggests that a given increment of rain occurring in April has a greater effect on protein content than an equal increment occurring in June, merely because rain is much scarcer in April. There can be little doubt that this hypothesis is essentially true, but the extent to which it serves to explain the difference between the effectiveness of April and June rainfall must await the results

of further investigation. In the meantime it should be borne in mind that the results of the present investigation stand independently of any explanation of them: under the conditions studied it appears that additional rainfall in April, early May, or late July, is more effective in reducing protein content than additional rainfall at other periods of the year.

An investigation of the sort reported in this paper presents many difficulties. The imperfections of the data are all too apparent and the propriety of analysing them by means of the selected statistical method may be open to question. Having accepted these difficulties, the application of the method leads inexorably to the production of a broad generalization, in this paper, the curve in Fig. 2. Like all generalizations it must be qualified, it applies only under certain conditions, it is not even fully representative of the original data since these contain various contradictions and exceptions not apparent in the final broad generalization. But the last difficulty is still the greatest. Having obtained the curve representing the effect of additional rainfall on protein content, it must still be interpreted. Whereas previous procedures are objective and mechanical, the last step is not. However, there is this to be said, that the responsibility for interpreting the curve can be shared with the reader.

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ARE THERE LIVING BACTERIA IN PERMANENTLY FROZEN SUBSOIL?[1]

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Abstract

Samples of frozen subsoil were obtained aseptically at Churchill, Man., located on Hudson Bay at about 58° north latitude. These were cultured on various media. The procedure followed failed to demonstrate the presence of viable micro-organisms in the permanently frozen zone of a clay subsoil. Bacteria were isolated from samples obtained as deep as nine feet from the surface of a coarse gravel subsoil.

Introduction

Soil is a place of life—a dynamic mass harbouring large populations of many different forms of micro-organisms. These minute living creatures feed upon the mineral salts of the soil and the remains of plants and animals, and produce physical and chemical changes of great importance to agriculture. They are most numerous in fertile soil and in its surface layer, where organic matter abounds. Early investigators were not in agreement as to the presence of living bacteria deep in the subsoil. More recent work has shown that bacteria and fungi can be isolated from samples taken at considerable distances from the surface. Brown and Benton (1), after presenting a list of workers who have reported on the occurrence of bacteria at different depths of soil, recorded numbers ranging from 2100 to 81,600 bacteria and from 11 to 325 fungi per gram of air-dry soil in the C_2 horizon of 29 profiles sampled. These represented different soil types and dates of sampling from virgin and cultivated soils in Iowa. The depth of this horizon varied between 24 and 50 in. In a somewhat similar study with virgin soil in Manitoba, Timonin (13) in 1935, reported counts from the *C* horizon ranging from 20,500 to 6,000,000 bacteria and from 40 to 1840 fungi per gram of moisture-free soil in 10 profiles sampled. In this case subsoil was obtained between depths of 17 and 44 in. in the various profiles. Further, this investigator presented counts of 48,000 and 63,500 bacteria and of 500 and 875 fungi, respectively, per gram of moisture-free soil in two samples of the heavy blue clay obtained 64 and 76 in. below the surface of a peat soil. In neither of the investigations referred to was any sample taken at these depths found to be free of bacteria or of fungi. Furthermore, the decrease in count from the horizon immediately above in each profile was relatively small, which would strengthen the belief that considerable numbers of these organisms exist at much greater depths, and in fact, as deep as the soil itself.

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Since there are vast areas where the subsoil is frozen and evidently has remained so for centuries, a study of these soils might be expected to reveal information of fundamental scientific interest in relation to (1) the possibility of micro-organisms withstanding long periods of dormancy and (2) the possible presence of types capable of carrying on normal metabolism in this frozen medium. Positive proof of dormancy would depend upon acceptance of the fact that the organisms were in the soil at the time it was frozen and upon suitable procedures for recovering them. It would be conditioned by satisfactory evidence that the chances of recent penetration by surface organisms and contamination due to faulty technique during sampling could be ruled out. A negative result, on the other hand, might mean merely that the portion sampled did not contain bacteria at the time of freezing. Or, it might mean that proper conditions for culturing the organisms in the soil were not provided in the procedure followed. A negative finding could be interpreted only in relation to the conditions of the experiment, and would have no bearing on that to be expected with another medium or set of incubation conditions. Obviously, a larger number of negative results, under different procedures, would have more weight than a single observation. Proof of a flora capable of reproducing in a frozen subsoil would involve the demonstration of reproduction in situ, which presents many difficulties. Or it would depend upon evidence that numbers of micro-organisms in one area changed significantly from time to time. This would necessitate working on samples immediately after obtaining them, or provision for transporting them under conditions that would prevent change in numbers during transit. Too, it would mean that the method used to estimate numbers of organisms in soil is capable of producing a result that can be accepted as correct.

The presence of micro-organisms in low temperature areas has been demonstrated. Darling (2) isolated many common species of spore-forming and non-spore–forming bacteria, as well as yeasts and moulds, from samples of soil and of snow collected during the second Byrd Antarctic expedition.

Issatschenko and Simakowa (4) reported on the bacteria of Arctic soils. They isolated several groups of bacteria common to temperate climates, and suggested that they might represent specific strains developed at low temperatures. Kazansky (6) studied the microflora of the soils of the Nova Zemblia, obtained at 73° 6′ north latitude. The author distinguishes seven types of soil in the area investigated. One of these, designated the flood-plain tundra, is found on a layer of permanent congelation and is not deeply embedded (20 to 40 cm.). It is almost black and of viscous consistence, with a good vegetative cover. Unfortunately, the data on numbers and groups of bacteria presented in his tables do not apply to this type of soil. This would have been of direct interest in the subject under consideration in this paper. He presents counts of bacteria by the microscopic method in profile studies on three of these types of soil, which confirm the belief expressed earlier that organisms exist as deep as the soil itself. Studies on various media reveal that the population consists of many kinds of bacteria, including

anaerobic and aerobic cellulose decomposers, nitrifiers and denitrifiers, nodule bacteria, and anaerobic nitrogen fixing bacteria. *Azotobacter* has not been detected. Levinskaya and Mamincheva (7) carried out an extensive study of the microbiological processes taking place in soils of Murmansk. Holes were dug to a depth of one metre in virgin soils, representing peat, sand, and sand–clay types. Samples from different depths were taken at one- to two-month intervals. Various media were used for the growth of different physiological groups of micro-organisms and for total counts. The results show that the population is small in these soils. It is highest in the sandy-clay soils (100,000 to 157,000 per gram of soil) and in samples obtained in the winter. The physiological processes are extremely slow and weak in intensity, showing a certain increase in the winter and autumn seasons. The authors attribute these evidences of low microbial activity to the low pH (5.2 to 6.4) of the soils investigated and to the influence of prolonged low temperatures. The population is smaller in the lower depths and the physiological activity weaker. The kinds of bacteria encountered are the same at all depths. *Azotobacter* was not detected in any of the studies. The same was true of members of the *Escherichia coli* group and the urea decomposing group. This prompted the authors to express the opinion that they had really selected virgin soils, that the micro-organisms isolated are inherent in soils of northern latitudes, and are cosmopolitans, adapting themselves to temperature, light, and soil conditions. Kapterev (5) reported on an investigation with frozen subsoil, carried on at 53° 58′ north latitude in Siberia. He took samples aseptically from the sides of pits and from borings in frozen subsoil, transferred them to distilled water and incubated them for several weeks. The presence of living organisms in the samples was demonstrated repeatedly in his experiments. These represented several genera of unicellular and filamentous algae, moss, and a lower crustacean.

Lipman (8–12) has done much to create interest in the possibility of bacteria existing for extreme periods of time under conditions where accepted ideas about bacterial metabolism could not apply. Within the last decade this investigator reported having found viable bacteria in pre-Cambrian rocks, anthracite coal, stony meteorites, and in ancient rock structures erected by man; he suggested that these organisms must have existed in a quiescent state for long periods of time,—even for millions of years. Since his claims represent a direct contradiction of much that is taken for granted about living organisms, his work has been subjected to critical examination.

Permanently Frozen Subsoil at Churchill, Man.

The subsoil at Churchill, Man., which is located on the Hudson Bay at about 58° north latitude, is frozen. The country is a vast plain, treeless for miles in some areas and supporting dwarfed trees—poplars, spruce, tamarack—and swamp bushes in others. The surface is marshy, the thick layer of moss providing insulation which prevents thawing for more than a few feet at the surface during the short summer seasons. Underneath is to be found heavy

clay, which is some places is not more than a foot below the surface, while in other areas the bog seems to be bottomless. Official data on the temperature of the subsoil in this locality are not available. Residents are satisfied that at no time in the past 20 yr. has the thaw exceeded three feet. The accompanying table* presents data on air temperature at Churchill, which add

TABLE I

TEMPERATURES IN DEGREES CENTIGRADE, CHURCHILL, MAN.

Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
						Mean						
1932	−24.4	−28.9	−24.4	−10.6	−0.6	6.1	12.2	16.1	7.2	−3.3	−13.3	−25.6
1933	−28.9	−28.9	−20.0	−12.2	−2.2	4.4	12.8	—	7.2	−3.9	−20.6	−31.1
1934	−26.7	−27.2	−22.2	−10.0	−3.9	4.4	12.8	10.0	4.4	−0.6	−12.2	−24.4
1935	−33.9	−20.6	−22.2	−10.0	2.2	4.4	11.7	11.7	5.6	−5.0	−18.9	−22.2
1936	−31.7	−30.6	−18.9	−15.6	−4.4	5.0	14.4	12.2	6.1	—	−16.7	−25.0
1937	−29.4	−23.9	−18.9	− 6.1	3.3	7.2	14.4	11.7	6.1	−1.7	−14.4	−25.0
1938	−24.4	−25.0	−17.8	−13.9	1.1	8.9	12.8	11.1	8.9	0.6	−14.4	−17.8
1939	−27.8	−31.7	−25.0	−10.0	−1.1	4.4	11.7	10.6	4.4	−5.6	−11.7	−16.1
1940	−21.1	−23.3	−18.9	−10.0	0.0	6.1	13.9	12.8	10.0	−1.1	−16.7	−23.3
1941	−26.1	−22.2	−18.9	− 9.4	−1.1	6.7						
						Maximum and minimum						
1932	− 5.0	−10.0	−11.1	3.3	24.4	22.2	30.0	28.9	20.0	6.1	3.3	− 5.0
	−38.9	−42.2	−35.6	−27.8	−21.1	− 2.8	− 0.6	6.1	0.0	−11.1	−31.7	−35.6
1933	−10.0	−13.9	5.0	6.1	12.2	22.2	27.8	—	22.2	7.8	− 3.3	−17.2
	−37.2	−41.1	−38.3	−27.8	−18.3	− 3.3	1.1	—	− 1.1	−20.6	−33.3	−42.2
1934	− 5.0	− 7.8	− 4.4	3.9	9.4	20.6	28.3	24.4	22.8	11.1	0.6	− 4.4
	−37.8	−38.3	−35.0	−28.9	−22.8	− 3.3	3.3	2.8	− 5.6	−14.4	−27.8	−43.9
1935	3.9	− 1.7	− 2.8	11.1	28.9	22.2	28.9	24.4	19.4	10.6	− 3.3	− 1.7
	−45.0	−35.0	−36.7	−29.4	−13.3	− 3.3	1.1	1.7	− 3.9	−18.9	−32.8	−36.7
1936	−15.6	−11.1	− 3.3	2.8	8.4	23.9	29.4	25.0	18.9	5.0	2.8	− 5.0
	−43.9	−43.9	−33.9	−31.7	−22.8	− 3.3	3.3	5.6	− 2.8	−22.8	−30.6	−42.2
1937	−16.7	− 0.6	− 1.7	10.6	20.0	26.7	27.2	25.6	22.2	8.4	− 2.2	−11.1
	−40.6	−37.8	−35.6	−25.0	− 8.4	− 1.1	3.9	0.0	− 1.7	−12.2	−27.8	−38.3
1938	− 8.4	− 2.8	2.2	6.1	18.9	30.0	27.8	22.2	22.2	16.7	− 0.6	− 1.7
	−37.8	−37.2	−31.7	−30.6	−10.6	− 3.3	3.9	3.9	− 0.6	−16.1	−28.3	−31.7
1939	−15.0	−16.7	0.0	5.6	17.8	23.3	26.1	25.0	18.3	7.2	2.8	1.1
	−38.9	−40.0	−40.0	−24.4	−12.2	− 4.4	2.2	0.0	− 3.9	−17.8	−25.6	−31.7
1940	0.0	− 7.8	− 3.3	5.0	21.1	18.3	25.0	28.9	28.9	9.4	0.6	− 9.4
	−37.8	−35.6	−37.2	−26.1	−16.7	− 2.8	5.6	2.8	0.6	−14.4	−30.0	−35.0
1941	− 7.2	− 3.9	− 2.8	7.2	12.2	26.7						
	−41.1	−36.1	−36.7	−30.6	−12.2	− 3.9						

* *Courtesy of Winnipeg office, Air Services, Meteorological Division, Department of Transport, Canada.*

weight to the opinion that the subsoil in this area has remained frozen for a long time. The flatness of the country, coupled with its swampy nature, makes the problem of obtaining samples of subsoil free from contamination difficult. Water seeps into any excavation made during the summer and carries with it types of micro-organic life characteristic of the surface layer. This difficulty could be obviated by working back from the face of a railroad cutting, where artificial drainage carries off the surface water. Only one such cutting could be found within 100 miles of Churchill. Since there is no other means of land communication to the port, the discovery of suitable sampling places is the first major difficulty to be overcome in any attempt to obtain valid information on the microscopic life in this district. Excavating heavy clay soil, frozen solid, in an area so far removed from industrial centres that the cost of machine digging or boring would be very high presents another serious problem. The hole must be large enough to permit working with aseptic technique while sampling, and deep enough to provide a fair margin below the thaw level. Recent penetration as a factor that might introduce microscopic life into frozen subsoil can be ignored. Surface water that seeps down to the frozen zone would freeze immediately and form a layer impervious to water. The subsoil beneath this could not be affected by the seepage.

Procedure

A clay cutting 8 ft. deep, located at Mile 505 on the Hudson Bay railway, was sampled on June 18, 1941. The clay was frozen immediately below the moss layer, which at this point was about 1 ft. thick. A pit 6 ft. deep and extending 8 ft. back from the base of the slope of the cutting was dug. Then a large chisel, sterilized over an open fire, was used to make horizontal excavations at three levels in the wall of the pit. Sterile pieces of steel, brought from the laboratory for the purpose, were used to dig about 10 gm. of soil from the top surface of an excavation and transfer it to a sterile test tube. Two samples were taken at each level, a different sampling tool being used for each. A fourth pair of samples was obtained from the clay immediately under the moss layer. As a check on the technique of sampling, soil sterilized in Petri plates in the laboratory was transferred to test tubes in the pit at approximately the same levels as the horizontal excavations. Further, plates containing sodium albuminate agar and others with Czapek's agar were exposed to the air of the pit for two minutes.

A second pit was dug on the slope of a gravel ridge rising about 50 ft. above the level of the surrounding country and extending for some miles parallel to the shore line of Hudson Bay. The ridge had been treed with spruce and was cleared only a few years ago, at the time of constructing the railway terminal and port. The sod and moss layer was about 2 ft. deep at the place excavated. Roots penetrated to a depth of 5 ft. The gravel was coarse and frozen in chunks, but not to a solid mass. It was readily loosened with an ordinary pick and a shovel. A thermometer inserted two inches into the wall at the 3 ft. level registered 2° C.; and at 6 ft. 0° C. This pit was

11 ft. deep and at the bottom extended back 12 ft. from the slope of the ridge. Samples were taken at four levels by the procedure described earlier.

The test tubes were packed carefully and transferred to the laboratory without refrigeration. Consequently, actual numbers of micro-organisms could have little significance because of the possibility of increase during the two weeks' exposure to summer temperatures. In order to minimize contamination that might take place in the laboratory during weighing, about half of each sample was transferred directly to a 50 ml. sterile dilution blank, the other portion being kept for further study. The initial dilution was shaken thoroughly, and a second dilution made by transferring 1 ml. to a 99 ml. blank. Transfers of 1 ml. were made in duplicate from each of the dilutions, representing approximately 0.1 and 0.001 gm. of soil, respectively, to the following media for culturing:

Difco nutrient agar in plates.
Sodium albuminate agar in plates; Med. 5 (3).
Amino acids–salts–glucose agar in plates (14).
Growth factors–salts–glucose agar in plates (14).
Nitrogen-free mannitol agar in plates; Med. 77 (3).
Sodium-nitrate–sucrose agar in plates; Med. 16 (3).
Peptone–glucose acid agar in plates; Med. 18 (3).
Difco nutrient broth in tubes, for protozoa.
Hay infusion in tubes, for protozoa.
Ammonium sulphate–cellulose solution in tubes; Med. 85 (3).
Glucose–peptone agar in deep tubes for anaerobes; Med. 12 (3).
Calcium nitrate solution (Detmer's) in tubes for algae; Med. 42 (3).
Thiosulphate* solution for sulphur oxidizing bacteria; Med. 65 (3).
Iron–ammonium* sulphate solution for iron oxidizing bacteria; Med. 74 (3).

Incubation was at 25° C. for one week for all agar plate studies and for protozoa, and for about one month for cellulose decomposers, anaerobes, sulphur oxidizing and iron oxidizing bacteria. The cultures for algae were incubated in a sunny window at summer temperature for six weeks. This procedure provided 24 results on the frozen subsoil and eight on the surface soil for each medium used with each type of soil studied. These represented duplicates from two dilutions from each of two samples taken at four different levels. The sterile soil transferred in the pit was studied in the same way, on the 0.1 dilution only.

Results from Excavation 1—Clay Cutting

Except for a few colonies, which clearly represented air contaminants since they did not appear on all plates in a set and were equally numerous from the 0.1 and 0.001 dilutions, all results for samples taken at depths of 54 and 40 in. were negative as were those also for 24-in. samples, except in the medium for anaerobic bacteria. All of the four transfers from 0.1 dilutions and one

** After transfers to the above media had been made about half of the remainder of the initial dilution was added to 50 ml. of each of these two media in 150 ml. Erlenmeyer flasks.*

from a 0.001 dilution produced colonies and gas in this medium. Samples taken at the 12 in. level, just below the moss where the soil was not frozen at the time of sampling, gave positive results in all media, except those for protozoa and iron oxidizing bacteria; counts ranged up to many hundreds of colonies per plate from the 0.001 dilutions on all agar media for bacteria, and up to 100 per plate from the 0.1 dilutions on media for fungi. Three transfers from 0.1 dilutions, but none from 0.001 dilutions, showed evidence of growth in the medium for anaerobic bacteria. The same number of transfers from 0.1 dilutions produced growth in the medium for algae. The activity of cellulose decomposers was evident in all tubes of media containing 0.1 dilutions, and in two of the four tubes containing 0.001 dilutions. The sulphur oxidizing medium supported motile short rods from all dilutions, the growth appearing white and flaky. All of the media containing transfers from the 0.1 dilutions of sterile soil, used to check the technique of sampling at the various levels in the pit, gave negative results. On the other hand, plates exposed two minutes to the air of the pit contained many colonies, in general appearance not unlike types of fungi and of bacterial colonies usually encountered as air contaminants.

Results from Excavation 2—Gravel Ridge

The results for this excavation were quite different from those for the clay cutting. Samples taken at a depth of 9 ft. produced many colonies, uncountable for the 0.1 dilutions and varying from 76 to 375 for the 0.001 dilutions on all plates prepared with the various agar media for bacteria. Two of the transfers from 0.1 dilutions gave positive results in the medium for anaerobic bacteria. In the media for cellulose decomposing bacteria, sulphur oxidizing bacteria, iron oxidizing bacteria, fungi, algae, and protozoa, there was no evidence of growth from any of the dilutions. The results for samples taken at 6 ft., 4 ft., and 1 ft. in general were in agreement with those at the 9 ft. level. Bacteria that developed on the plating media were more numerous in the higher level samples. A few fungi and cellulose decomposing bacteria were found in the samples taken at a depth of 4 ft. The 1-ft. level samples contained about the same numbers of organisms as the surface samples taken from the excavation in clay soil.

Discussion

The apparent discrepancy in the findings from the two excavations may be attributed to the difference in permeability of the two types of subsoil. Undoubtedly, the loose textured coarse gravel would permit thawing and seepage of surface water to a greater depth than would clay subsoil. The presence of bacteria in samples of the gravel subsoil cannot be accepted as an indication of their ability to withstand long periods of dormancy in a frozen medium. The organisms may have been carried there relatively recently in seepage water. The failure to demonstrate the presence of fungi in these samples weakens this statement, since one would expect fungal spores to be

found in contamination from surface water. The data published by Brown and Benton (1) and Timonin (13) indicate that the proportion of fungi to bacteria in the *C* horizon of unfrozen subsoil may not differ widely from that in the surface layer of the same profile.

It is obvious that a statement on either of the theories that prompted this study is not justified by the results obtained. This report merely presents some of the problems encountered and the findings obtained in a preliminary investigation.

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STUDIES IN TREE PHYSIOLOGY

III. THE EFFECT OF THE METHOD OF CUTTING ON THE WATER CONTENT OF TWIGS. A NOTE ON A PAPER BY McDERMOTT[1]

By R. Darnley Gibbs[2]

Abstract

Significantly higher water contents are found in short samples of twigs isolated by simultaneous cuts than in samples isolated by consecutive cuts in which displacement of water obviously takes place. This is in agreement with the results of McDermott. Natural water gradients are small and do not invalidate McDermott's figures. Displacement is not confined to the immediate neighbourhood of the cut.

Introduction

In a previous paper (2) the author concluded that most of the available evidence is in favour of the tension hypothesis of the ascent of sap in trees. If it *is* true that columns of water are drawn up from above then they must be under tension. That this is indeed the case seems to be borne out by observations on the rapid movements of dyestuffs introduced into newly-cut vessels and perhaps by the diametral changes associated with fluctuations in transpiration, though an alternative view—that vessels may at times contain gas at less than atmospheric pressure, rather than water under tension—has been put forward by Priestley (4).

It is clear that whether vessels contain water alone under tension or water and gas under reduced pressure, there will be a movement of water when the vessels are cut. The amount of movement, however, might differ greatly in the two cases. In the former an extremely small change might relieve the tension (since water is practically inextensible) unless the vessels are greatly contracted by the tension. Their expansion would then withdraw a considerable amount of water from the cut ends. If many of the vessels contain gas under reduced pressure cutting would be followed by the entrance of air but not by any great movement of water from near the cut end, unless individual vessels have both gas *and* water in them.

In early determinations of water contents (1) the danger of considerable change during cutting was regarded as a distinct possibility and some attempt to eliminate it was made by removing small cores of outer sapwood with a hollow punch. This, driven into the tree with a single heavy blow, isolated a short cylinder of wood without possibility of vertical displacement. Results from this led to the belief that changes were too small to affect seriously the determinations, but no extensive study of this was made.

A recent paper by McDermott (3) has reopened the question. McDermott isolated small sections from twigs by two simultaneous cuts, thus preventing

[1] *Manuscript received November 17, 1941.*
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withdrawal of water. He compared the water contents of such pieces with those of adjacent parts of the twigs taken by consecutive cuts both above and below the initial cuts. Significantly lower water contents were obtained for the latter. He concluded that these lower values were due to the displacement of water resulting from the release of tensions.

Although his results seem convincing he appears to have neglected one perhaps remote possibility—namely, that the natural gradient of water content in a twig might be such that (especially if single internodes were used) the middle piece would contain more water than the adjoining distal and proximal pieces.

It is not absolutely clear from McDermott's work that he used more than one internode in every sample, but his three samples were each about 3 in. long which would seem to make this certain.

Materials and Methods

In the present work, twigs of *Tilia*, *Ulmus*, and *Fraxinus* were used. In *Tilia*, samples were taken from single internodes of twigs of the current year's growth (Fig. 1*b*). The twigs of *Ulmus* and *Fraxinus* were older and individual samples extended over more than one internode, although they were about the same length as those of *Tilia* (about 1 in.).

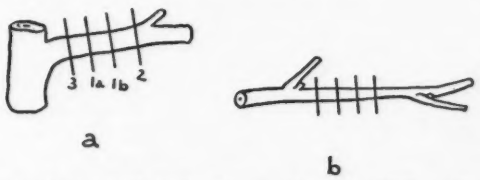

FIG. 1. *Diagrams showing methods of taking samples. (a) McDermott's method: middle piece isolated by simultaneous cuts, proximal and distal pieces removed by consecutive cuts. (b) Present method with Tilia: proximal, middle, and distal pieces are from a single internode.*

As in McDermott's work three samples were taken from each twig, but in order to eliminate any possibility of error due to existing gradients, the cuttings were made in such a way that sometimes the distal, sometimes the middle, and sometimes the proximal samples were made with simultaneous cuts, the others being made with consecutive cuts. No more than a few seconds elapsed between the initial cuts and the final bottling of all three samples in weighed rubber-stoppered tubes.

The first two experiments (B.G. 41.3 and 41.5) were carried out in the early afternoon, using twigs from young trees of *Fraxinus americana* L. and *Ulmus americana* L., growing in the Montreal Botanical Garden. The weather was hot and sunny and tensions presumably high, though no check of this was made. The results are summarized in Tables I and III.

McDermott carried out some experiments in which samples were taken on a dull, moist day, when transpiration presumably was slow and tensions

TABLE I

WATER CONTENT (%) OF TWIGS OF *Ulmus americana* AND *Fraxinus americana*; SAMPLES TAKEN FROM DISTAL, MIDDLE, AND PROXIMAL POSITIONS*

Twig	*Ulmus americana:* 1 to 2 p.m., July 14, 1941. (Expt. B.G. 41.3)			*Fraxinus americana:* 2 to 3 p.m., July 24, 1941. (Expt. B.G. 41.5)		
	Distal	Middle	Proximal	Distal	Middle	Proximal
1	**65**	63	64	**65**	63	62
2	71	**73**	69	58	**61**	56
3	77	76	**78**	66	65	**64**
4	**74**	70	70	**65**	59	58
5	73	**76**	71	59	**60**	56
6	78	76	**79**	65	63	**65**
7	**94**	86	87	**67**	62	60
8	72	**73**	72	69	**70**	67
9	71	69	**70**	62	63	**59**
10	**76**	71	74	**62**	67	61
11	70	**75**	65	61	**63**	60
12	81	80	**85**	68	67	**70**
Means	75.2	74.0	73.7	63.9	63.6	61.5
Means: samples taken with simultaneous cuts			**76.5**			**64.25**
Means: samples taken with consecutive cuts			73.2			62.4

** Figures in bold-face type are from samples made with simultaneous cuts; the others from samples obtained with consecutive cuts. Each twig came from a separate tree. All results are calculated on a dry-weight basis.*

small. He obtained significantly smaller differences in water content than under conditions of rapid transpiration. In view of McDermott's results, further experiments, D.H. 41.5 and 41.6, were carried out. Since at night transpiration is reduced, and tensions probably are lower, one set of observations was made in the early afternoon of a warm, clear day and another set at dawn the next morning. Single internodes from current growth on young trees of *Tilia americana* L. were used. The results are summarized in Tables II and III.

Results and Discussion

The results of experiments B.G. 41.3 and 41.5 are summarized in Tables I and III. Two facts are indicated by the figures given.

1. There appears to be a slight gradient in the water content of twigs of both trees, the distal samples containing more and the proximal samples less water than the middle pieces. While these gradients are not both statistically significant when considered separately, they are significant when considered as a group with the similar results from *Tilia* (P is <0.001).

2. Significantly higher values are obtained when samples are taken with simultaneous cuts than when they are made with consecutive cuts. This is in agreement with McDermott's findings.

The results of experiments D.H. 41.5 and 41.6 are summarized in Tables II and III.

TABLE II

COMPARISON OF WATER CONTENT (%) OF TWIGS OF *Tilia americana* IN EARLY AFTERNOON AND AT DAWN; SAMPLES TAKEN FROM DISTAL, MIDDLE, AND PROXIMAL POSITIONS*

Twig	*Tilia americana*					
	1 to 2 p.m., Sept. 15, 1941. (Expt. D.H. 41.5)			5 to 6 a.m., Sept. 16, 1941. (Expt. D.H. 41.6)		
	Distal	Middle	Proximal	Distal	Middle	Proximal
1	**123**	113	109	**136**	117	113
2	112	**129**	107	123	**125**	111
3	112	110	**120**	122	117	**123**
4	**132**	115	111	**136**	115	108
5	106	**121**	103	105	**107**	94
6	115	112	**120**	129	127	**131**
7	**99**	86	86	**122**	104	102
8	117	**130**	117	124	**129**	113
9	114	112	**120**	137	130	**132**
Means	114.4	114.2	110.3	126.0	119.0	114.1
Means: samples taken with simultaneous cuts			**121.6**			**126.8**
Means: samples taken with consecutive cuts			108.7			116.2

* *Figures in bold-face type are from samples made with simultaneous cuts; the others from samples obtained with consecutive cuts. Each twig came from a separate tree. All results are calculated on a dry-weight basis.*

TABLE III

ANALYSIS OF VARIANCE

Tree	Source of variance	D.f.	Sum of squares	Variance	*F*	*P*
Ulmus	Between twigs	11	1385.89	125.99	49.41	$\ll 0.01$
	Between positions	2	14.89	7.44	2.92	> 0.05
	Simultaneous vs. consecutive cuts	1	88.89	88.89	34.86	$\ll 0.01$
	Residual (error)	21	53.55	2.55		
Fraxinus	Between twigs	11	363.33	33.03	11.67	$\ll 0.01$
	Between positions	2	41.17	20.58	7.27	< 0.01
	Simultaneous vs. consecutive cuts	1	28.12	28.12	9.94	< 0.01
	Residual (error)	21	59.37	2.83		
Tilia (afternoon)	Between twigs	8	1976.67	247.08	53.71	$\ll 0.01$
	Between positions	2	96.22	48.11	10.46	< 0.01
	Simultaneous vs. consecutive cuts	1	988.17	988.17	214.82	$\ll 0.01$
	Residual (error)	15	68.94	4.60		
Tilia (dawn)	Between twigs	8	2086.96	260.87	74.75	$\ll 0.01$
	Between positions	2	642.74	321.37	92.08	$\ll 0.01$
	Simultaneous vs. consecutive cuts	1	675.57	675.57	193.57	$\ll 0.01$
	Residual (error)	15	52.36	3.49		

As in *Fraxinus* and *Ulmus*, a gradient appears to exist, with slightly higher water contents at the distal end. Significantly higher figures for water contents were also obtained from samples made with simultaneous cuts than from those made by consecutive cuts. This was true of the twigs cut at dawn as well as of those cut in early afternoon, though the difference was (on the average) slightly smaller at dawn.

A question that arises from work of this kind is—how far from a cut does displacement have measurable effect? An analysis of the figures given in the tables shows that, where the sample made with simultaneous cuts was the end one (either distal or proximal), of the three pieces taken it had a higher water content than the other two pieces; the latter, however, had almost exactly the same water contents (average for all species: 90.95% for the pieces next to the first piece and 91.07% for the remote pieces). It is true, of course, that the first figure is obtained from distal and proximal samples only, while the second is from distal, proximal, *and* middle pieces. That this does not affect the result is evident from the fact that the average water content of *all* distal and basal pieces taken together is almost exactly the same as that for *all* middle pieces (92.4 and 92.7%, respectively).

Acknowledgments

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STUDIES ON ROOT ROT OF CORN IN ONTARIO[1]

By J. K. Richardson[2]

Abstract

Root rot of corn in Ontario is caused primarily by parasitic soil microorganisms, the most important of which are species of *Pythium*, *Helminthosporium*, and *Fusarium* in that order. The disease causes a decrease in the stand by pre-emergence killing and a dwarfing of the plants by the parasitic invasion and destruction of their roots by the organisms. The pathogens have different optimum soil temperatures but the lower ranges favour those that cause the most severe damage. The roots of other field crops can be parasitized by the organisms found associated with corn root rot, but their effect on the development of the crop varies greatly. It has been proved under greenhouse conditions that the severity of the disease is greatly reduced if the corn is preceded by cover crops of soybeans and materially increased when preceded by timothy. Other crops tested have an intermediate effect.

Introduction

In some seasons, root rot may be a limiting factor in the growth of corn. In affected fields the stand is usually thin and the growth very uneven. By midsummer most of the plants are dwarfed and chlorotic. The examination of diseased plants reveals root systems rotted to such an extent that only a few secondary roots are able to function.

The present paper deals with laboratory and greenhouse studies of parasitic root rots of corn caused by species of *Pythium*, *Helminthosporium*, and *Fusarium*.

Literature Review

The earlier conceptions of corn root rot were that the organisms that caused stalk and ear rots were also able to infect and rot the roots when introduced into the ground by means of diseased seed. This theory was advanced in the Central States by Holbert and Hoffer (10) in 1920 and also by Koehler and his associates (15) in 1924, the latter stressing the fact that since corn and wheat are both subject to the ravages of *Gibberella Saubinetii* (Durieu & Mont.) Sacc., these crops should not follow each other in a rotation.

In 1923-24, Hoffer and Carr (9) working in Indiana, and Manns and Phillips (16) in Delaware, were convinced that the root rot condition in corn was associated with soil deficiencies or poor drainage and lack of fertility.

In Connecticut, in 1919, Clinton (3) observed oospores of a fungus, which he thought resembled *Phytophthora Cactorum* (Lebert & Cohn) Schr., in the bases of corn stalks affected with root rot, but did not isolate the fungus. Johann and her co-workers in Illinois and Wisconsin in 1926 (11, 12)

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attributed the disease to infection by a species of *Pythium*, later described by Drechsler (5) as *Pythium arrhenomanes* n. sp. Within the next several years Valleau *et al.* (21) and Branstetter (1) described similar root rots of corn and stressed their similarity to a root rot of sugar-cane in Hawaii, which was described by Carpenter (2) in 1921 and attributed by him to soil inhabiting *Pythium*-like fungi.

Melhus *et al.* (17) and Wen-Chun Ho and Melhus (8) in their recent researches on maize root rot in Iowa described the symptoms following infections by *Pythium graminicola* Subr. and *P. de Baryanum* Hesse and classified a large number of isolates in order of their importance as root parasites; among them *P. graminicola* and *Rhizoctonia Solani* Kühn were considered to cause severe injury, while *P. de Baryanum* and *Helminthosporium sativum* P. K. & B. were of lesser importance. Recently Wen-Chun Ho (7) suggested that there appears to be a succession of invasions by different species of fungi as the season advances. He also found a variation in the susceptibility of varieties of maize to some of the pathogenic organisms.

Materials and Methods

Corn seed after being selected for uniformity and freedom from disease was disinfected in a 1 : 1000 mercuric chloride solution and planted in steam sterilized and non-sterilized soil of three types (1) clay loam that had produced a badly rotted crop of corn, (2) light sandy loam on which brown root rot of tobacco had been severe, and (3) greenhouse compost. All unsterilized soils produced crops with typical root rot symptoms, while the corn growing in steamed soil was entirely free from the disease (Fig. 6).

Isolations were made from incipient infections on small roots of corn both from the field and from root rot soil in the greenhouse. These isolates were then tested on corn growing in artificially inoculated soil, to determine their parasitic capabilities.

Throughout the experiments inoculations were made by planting seed in sterilized soil in which was incorporated an inoculum, in volume equal to 5 % of the soil, of a pure culture of the isolates growing on a mixture of crushed oats and corn-meal.

Root sections for microscopic examination were taken from young incipient infections, cleared by heating in a solution of lactophenol (to which a small quantity of basic fuchsin had been added) and mounted in clear lactophenol.

Isolations and Preliminary Observations

By selecting incipient root infections for microscopic examination it was possible, in most instances, to identify the parasitic forms and obtain a fairly accurate conception of the flora present. In the diseased roots of corn were found a number of distinct types of organisms, many of which are commonly present in diseased roots of other species of plants. *Pythium* spp. in the form of lobulate sporangia and oospores and fine septate mycelium were most

commonly observed. The so-called phycomycetous mycorrhizal fungus (6, 13, 23) which formed a profusion of mycelial growth on the root surfaces, as well as arbuscule and vesicle development within the host tissues, was also in evidence. *Olpidium Brassicae* (Wor.) Dangeard (6, 19, 22) occurred frequently, often in rootlets free from visible discoloration. Both parasitic and saprophytic species of nematodes, mycelium of *Helminthosporium* and *Rhizoctonia* spp., mycelium and chlamydospores of *Fusarium* spp., fungi that could not be classified, as well as numerous areas of necrotic tissue apparently devoid of organisms, were also present.

In a preliminary series of inoculations on corn in artifically inoculated soil, involving all the organisms isolated from corn roots, only six proved to be definitely parasitic. These were a *Pythium* of the *Nematosporangium* group, probably *P. arrhenomanes* Drechs., *P. de Baryanum* Hesse, *Helminthosporium bicolor* Mitra, *H. Maydis* Nisikado & Miyake, and two species of *Fusarium*. These parasitic forms, which caused various degrees of injury up to severe necrosis and distintegration of root tissue, were used in subsequent experiments. Careful examinations of corn roots infected with pure cultures of the above organisms made it possible to determine the specific symptoms produced by each pathogen (Figs. 7 to 11) and the appearance of the organisms within the host tissues. These observations provided a basis for future examinations of the roots of corn and other host plants growing in naturally infected soil where the specific pathogenic factor was not known.

The Effect of Pathogens Isolated from Corn Roots on the Germination and Growth of Corn

Flats of sterilized soil were artificially inoculated with pure cultures of the above-mentioned organisms, seeded to corn, and kept at a temperature of 65° to 75° F. *Pythium arrhenomanes* and both *Helminthosporium* species affected the germination, causing a reduction of from 40 to 50% and 5 to 10%, respectively. In plants that developed, the leaf growth varied in direct relation to the degree of root damage induced by the pathogen in question. After 24 days, plants in sterilized compost and those in the flats inoculated with *Fusarium* spp. were from 12 to 14 in. high, those to which *P. de Baryanum* and sterile culture medium had been added were 8 to 11 in., while with *P. arrhenomanes* and *Helminthosporium* spp. growth was only 5 to 8 in. in height. In addition to the reduction in growth many plants in the flats inoculated with *P. arrhenomanes* were chlorotic and malformed, while a number of those inoculated with *Helminthosporium* spp. were infected above the ground on the coleoptile or first leaf.

In experiments in which sterilized culture medium was added as a control, there was invariably a retardation in growth, equivalent to, or even slightly more than where weak or moderate parasites had been used. This cannot be attributed to chance infection since the roots of such plants, though slightly less vigorous, were as free from discoloration as those growing in steamed soil. On the other hand, where non-parasitic isolates were used as inoculum,

both leaf and root growth were comparable to plants from the steamed soil series (Figs. 4 and 5).

The Effect of Parasitic Fungi Isolated from Corn Roots on Other Host Plants

Since there was a marked similarity in organisms found in the roots of corn growing in different soils, and it has been shown (6) that the same species of fungi may attack the roots of a variety of hosts, 11 field crops were tested for their susceptibility to infection by the pathogens isolated from corn to determine those best suited for rotation with that crop.

Wheat, oats, rye, timothy, soybeans, red clover, alfalfa, rape, buckwheat, sugar beets, tomatoes, and corn were grown in soil artificially inoculated with pure cultures of the fungi, and in addition to macroscopic observations on plant growth and root condition, careful microscopic examinations were made to determine the variation in parasitism on the respective host roots.

All plants were kept under similar environmental conditions in the greenhouse and although the artificially inoculated soil might tend to exaggerate the infection, particularly of the weaker pathogens, nevertheless the results

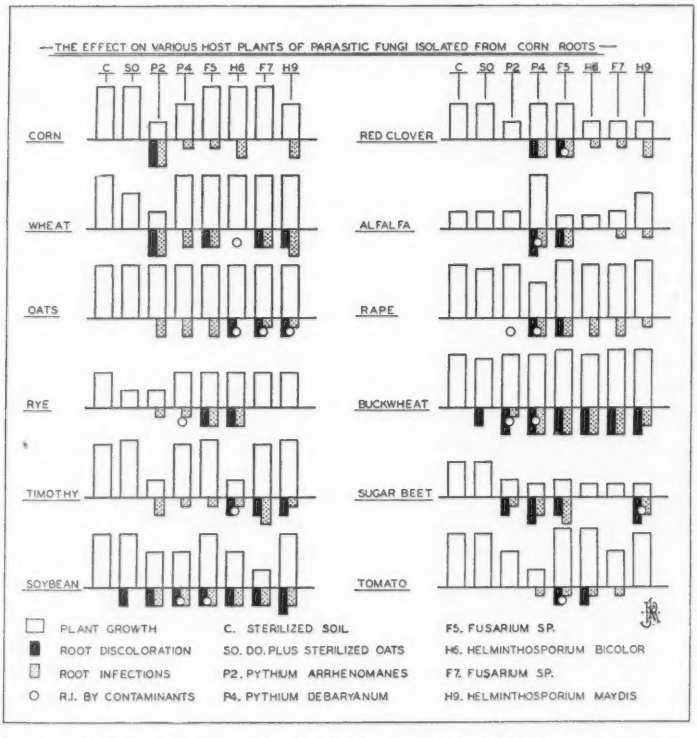

FIG. 1. *The effect on various host plants of parasitic fungi isolated from corn roots.*

obtained may be regarded as characteristic of the parasitic capabilities of the isolates tested. In Fig. 1 the condition of plants growing in sterilized soil (*C*) was taken as normal for the environment under which the experiment was conducted.

In corn, wheat, timothy, and sugar beet affected by P_2 and other plants in which affected root systems and significant reduction in growth coincided, there can be little doubt that the organisms concerned were responsible for the stunting. On the other hand in soybeans and buckwheat, for example, where there were severely discoloured and infected roots without material reduction in growth, the effect of the organisms may be considered as negligible.

Micro-organisms in the Roots of Various Crop Plants When Grown on Corn Root Rot Soil

Following the determination of the reactions of the pathogens isolated from corn roots on different host plants growing in artificially inoculated sterilized soil, a test was conducted to study the behaviour of these same hosts when growing on soil that had produced severe root rot of corn in the field similar to that illustrated in Fig. 3. No attempt was made to grow the plants to maturity, but periodic examinations were made of typical root lesions from each host to record the types of micro-organisms present.

Table I gives the general macroscopic appearance of the various plants as well as a record of the microscopic condition and fungus flora of their roots. It is reasonable to assume that similar conditions would prevail when these crops are grown on infected soil under natural field conditions.

Pythium spp., as shown by the presence of lobulate sporangia and oospores (1 and 2) and some of the mycelial invasion recorded (7), were the organisms most prevalent in the roots. Where this invasion is accompanied by root discoloration and abnormal growth, as in corn, wheat, timothy, alfalfa, and buckwheat, there can be little doubt that these crops when grown either immediately before or following corn, would tend to aggravate the root rot situation. It is interesting to observe that although no *Rhizoctonia* spp. were isolated from corn roots, these fungi must have been present in large numbers in corn root rot soil since in the present experiment when damping-off was evident, *Rhizoctonia* mycelium was observed in the roots of many of the plants. The other micro-organisms, although sometimes present in considerable quantities, do not appear to have any appreciable effect on the crop.

Cover Crop Experiments

Following the significant variation in the reactions of different crop plants when grown on root rot soil, two similar series of experiments were conducted in which four successive crops of each of 12 host plants in addition to five different four-crop rotations of groups of these same plants were grown (1) in corn root rot soil and (2) in steamed compost inoculated with a mixture of pure cultures of corn root pathogens. The leafy succulent growth from

TABLE I

THE DEVELOPMENT AND ROOT FLORA OF VARIOUS PLANTS WHEN GROWING IN CORN ROOT ROT SOIL

Host	Condition of plants	Condition of roots											
		Macroscopic			Microscopic								
		A	*B*	*C*	1	2	3	4	5	6	7	8	9	10
Corn	Stunting of growth	−	+	−	+	+	+	−	+	+	+	+	−	−
Wheat	Chlorosis of leaf tips	−	−	+	+	+	−	−	−	+	−	−	+	−
Oats	Normal growth	+	−	−	−	−	−	+	−	−	−	−	−	−
Rye	Normal growth	−	+	−	+	−	−	−	−	−	+	−	−	+
Timothy	Considerable damping-off	−	+	−	+	+	+	+	−	−	−	−	−	−
Soybeans	Normal growth	−	+	−	−	−	+	−	−	−	+	−	−	+
Red clover	Some damping-off	+	−	−	+	+	−	−	−	+	+	+	−	+
Alfalfa	Severe damping-off	−	+	−	+	+	−	+	−	−	−	+	−	−
Rape	Some damping-off	+	−	−	−	−	−	−	−	−	+	+	−	−
Buckwheat	Fair growth	−	−	+	+	+	+	−	−	−	−	−	−	−
Sugar beets	Severe damping-off	+	−	−	−	−	−	+	−	−	−	−	−	−
Tomatoes	Slight damping-off	+	−	−	−	−	−	+	−	−	+	+	+	−

NOTE.— *A* = *apparently healthy roots, no discoloration.*
B = *slight discoloration or a few lesions.*
C = *severe browning or many lesions.*

1 = *Pythium, lobulate sporangia.*
2 = *oospores.*
3 = *Helminthosporium mycelium and spores.*
4 = *Rhizoctonia mycelium.*
5 = *Olpidium brassicae.*
6 = *mycorrhizal fungus.*
7 = *miscellaneous fungus invasion (not identified).*
8 = *nematodes and eggs.*
9 = *isolated necrotic areas.*
10 = *cell breakdown without visible fungus.*

each crop was thoroughly incorporated with the soil in which it had grown and initial decomposition was allowed to occur before the succeeding crop was sown. Sixteen days after the incorporation of the last cover crop, corn was planted in each of the pots.

A complete record of the condition of the corn following each of the cover crops and rotations was made for each of the quadruplicate series of pots with both types of soils. In general there was a marked similarity in the behaviour of the corn on both the naturally and artificially infected soils. The growth of all the host plants was more vigorous on the steamed compost and there was a slightly greater variation in the diseased condition of the roots. Since the differences between the two series were entirely quantitative, only the averages of the groups growing in naturally infected soil will be considered.

Though it was impossible to obtain accurate numerical counts of the diseased roots, it was possible to obtain a reasonably accurate idea of the condition of the corn roots as a whole, as affected by the various preceding

crops. Numerical ratings were assigned to different degrees of infection according to the following schedule.

1. Only a few visible lesions on root laterals.
2. Slight infections on a number of laterals.
3. General infection on most laterals.
4. Most laterals infected and some destroyed.
5. Severe infection of laterals and lesions on the primary radicle.

These data and the corresponding height of plants are recorded in Table II. See also Figs. 12 to 14.

TABLE II

THE EFFECT OF VARIOUS COVER CROPS ON THE SUBSEQUENT DEVELOPMENT OF CORN IN ROOT ROT SOIL

Successive crops			Rotations		
Preceding crops	Height, in., 18 days	Root infection rating	Preceding crops	Height, in., 18 days	Root infection rating
Soybeans	11.0	0	Rape Rye Timothy Soybeans	13.0	1
Red clover	11.0	3			
Oats	9.5	1	Soybeans Wheat Alfalfa Sugar beets	10.5	2
Wheat	9.5	2			
Alfalfa	9.0	2			
Rye	9.0	3	Oats Soybeans Rape Alfalfa	10.5	3+
Tomatoes	8.5	1–			
Corn	8.0	5			
Buckwheat	8.0	2	Buckwheat Red clover Timothy Wheat	8.5	3
Rape	7.5	1			
Sugar beets	7.5	2	Red clover Buckwheat Oats Timothy	7.5	5+
Timothy	7.0	4			

Specimens of typically affected corn rootlets from each series listed in Table II were also examined to determine possible variations in their microflora. Although a comparatively small number of roots were examined following each series of cover crops, the type and quantity of material examined was comparable in each.

In the graph, Fig. 2, the size of the blocks for each particular type of invasion is proportionate to the total amount of infection in the roots examined. In the case of nematodes, the actual number present was recorded. Only the organisms within the host tissues were taken into consideration and

no attempt was made to differentiate between primary and secondary invaders.

In the root examinations no differentiation was made between the species of *Pythium* observed. Both *P. arrhenomanes* and *P. de Baryanum* were present, but the former was responsible for the greater number of infections. The oospores were also predominately *Pythium* spp., though it is conceivable that *Phytophthora* spp. may also have been present. It was difficult to positively identify much of the fine mycelium present, but a comparison with the infections produced in the pure culture inoculations previously recorded leaves little doubt that the majority of these could be attributed to invasions by *Fusarium* spp. No classification was made of the nematode populations, both parasitic and saprophytic forms being present.

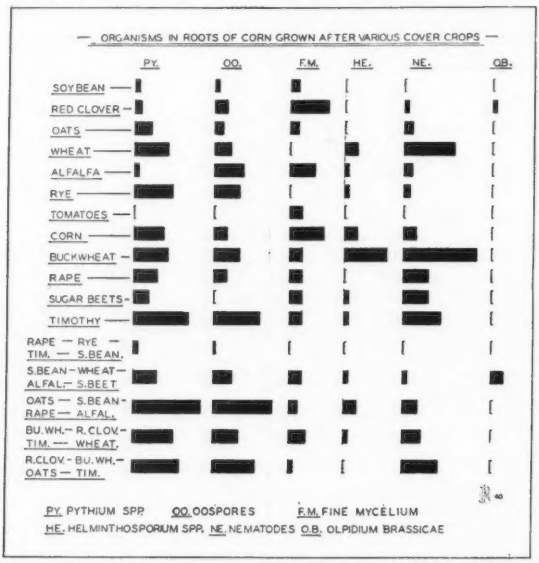

FIG. 2. *Organisms observed in the roots of corn grown in root rot soil following various cover crops.*

In a comparison of the root infection rating in Table II with the organisms observed in the roots as recorded in Fig. 2, it will be noted that in most roots the macroscopic condition is reflected in the amount of infection present as recorded from microscopic examination. In general, however, the records of microscopic examinations convey a somewhat erroneous impression, since the amount of infection present appears greater than the macroscopic root infection rating would indicate. In making microscopic examinations it was not possible to take into account the severity of infection for while similarly infected roots were examined it was sometimes necessary to examine the worst and sometimes the best of the diseased roots. For instance, when

corn followed soybeans, it was extremely difficult to find infected rootlets but in corn following timothy or corn, the difficulty was to find tissue that was not too severely infected for comparable examination. It is fully realized

FIG. 3. *Field of corn severely affected by root rot.*

FIG. 4. *Corn after 24 days' growth in steamed compost inoculated with (1) Pythium arrhenomanes, (2) P. de Baryanum, (3) sterile culture medium (oats plus corn-meal), (4) not inoculated.*

FIG. 5. *Same as Fig. 4, inoculated with (1) Helminthosporium Maydis, (2) H. bicolor, (3) a non-parasitic isolant, (4) not inoculated.*

that with the majority of specimens a very small percentage of affected rootlets was examined and additional examinations might have increased the preponderance of certain types of infections. However, since similar tissue was examined in all instances, it is reasonable to assume that a fairly accurate idea of the organisms responsible for the root damage was obtained.

This experiment shows beyond any doubt that the general vigour and condition of a crop of corn growing on root rot soil is influenced materially by the crops that precede it as indicated by the growth following successive plantings of the same crop. The most significant effect, however, appears to be produced from the crop immediately preceding, as demonstrated in the crop rotation series.

There is undoubtedly a certain stimulation derived from the growth of leguminous crops, such as soybeans, red clover, and alfalfa, but this advantage is not necessarily reflected in the condition of the roots. Following soybeans the roots of corn appear perfectly healthy and it was difficult to find even a trace of decay, whereas after red clover or alfalfa the roots were little cleaner than those grown in soil in which corn had been grown continuously. In contrast, timothy had the most severe detrimental effect, the reduction in growth and diseased condition of the roots being more severe than when corn had been grown continuously. All other crops tested had an effect intermediate between these two extremes. It is interesting to note that whatever the beneficial effect of soybeans on the following corn crop may be, it is an extremely potent factor, since in the rotation ending with this crop, in spite of the fact that it was immediately preceded by timothy (the most detrimental of the crops tested), the corn following this rotation was the best of any of the five tested.

Since it has been shown that the roots of soybeans growing in artificially inoculated soil are as susceptible to invasion by corn root rot pathogens as those of timothy, the results of these experiments seem to indicate that some factor other than parasitism must be involved in the beneficial or detrimental effects derived from the various crops tested.

Although all experiments were conducted under greenhouse conditions and it is fully appreciated that the results obtained may be abnormal to a degree, nevertheless it seems reasonable to assume that a similar though perhaps less pronounced effect might be expected when these same crops are grown in the field.

FIG. 6. *Root systems of corn plants after 10 weeks' growth in root rot soil (left) steam sterilized, (right) not steamed.*

FIGS. 7 and 8. *Corn seedlings with roots destroyed by P. arrhenomanes.*

FIG. 9. *Root lesions caused by P. de Baryanum.*

FIG. 10. *Typical root lesions caused by Helminthosporium spp.*

FIG. 11. *Corn roots infected by Fusarium spp. showing slight discoloration, laterals absent on infected areas.*

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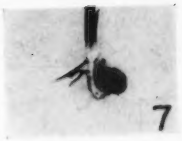
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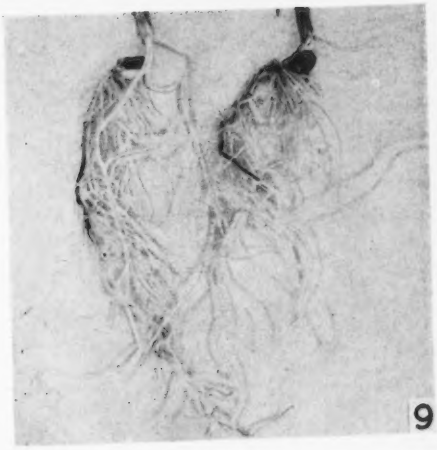
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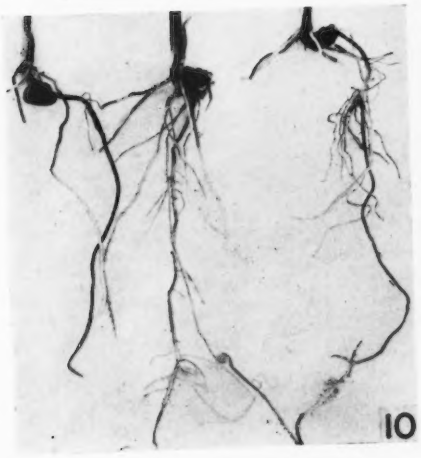
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TABLE III

THE EFFECT OF ROOT ROT PARASITES ON CORN GROWING AT CONTROLLED SOIL TEMPERATURES AND WATER HOLDING CAPACITIES

Temp., °C.	W.H.C.*, %	Inoculum	Germination			Growth in four weeks	
			Began, days	Ended, days	%	Leaf length, in.	Wt. of 100 plants, oz.
9 to 11	60	Control	10	20	96	5.0	1.6
		Sterile medium	10	21	96	4.5	1.6
		Fusarium	10	19	96	5.0	1.5
		Pythium	—	—	0	—	—
		Helminthosporium	11	23	28	Died	—
	90	Control	10	19	92	3.5	1.1
		Sterile medium	10	16	84	3.0	0.9
		Fusarium	11	22	94	4.0	1.2
		Pythium	—	—	0	—	—
		Helminthosporium	11	17	14	Died	—
13 to 15	60	Control	6	11	96	13.0	8.6
		Sterile medium	7	13	100	11.0	6.0
		Fusarium	7	12	98	12.0	7.1
		Pythium	8	11	4	2.8	—
		Helminthosporium	7	13	76	3.0	0.09
	90	Control	7	12	100	16.0	10.0
		Sterile medium	6	13	100	11.0	6.0
		Fusarium	6	14	86	16.0	8.8
		Pythium	8	15	4	2.0	—
		Helminthosporium	6	14	68	5.0	1.9
17 to 19	60	Control	5	8	100	19.0	16.6
		Sterile medium	5	8	96	20.0	15.6
		Fusarium	5	7	98	18.0	12.2
		Pythium	6	12	46	5.0	3.5
		Helminthosporium	5	10	94	13.0	6.2
	90	Control	5	7	100	24.0	21.0
		Sterile medium	5	7	96	19.0	12.5
		Fusarium	5	11	90	23.0	17.1
		Pythium	5	9	30	6.0	3.5
		Helminthosporium	5	10	94	17.0	9.9
22 to 24	60	Control	4	5	96	20.0	17.7
		Sterile medium	2	6	100	20.0	13.5
		Fusarium	3	9	100	21.0	13.3
		Pythium	4	9	86	7.5	2.5
		Helminthosporium	4	9	98	19.0	9.3
	90	Control	3	6	100	26.0	26.0
		Sterile medium	4	5	100	24.0	19.0
		Fusarium	3	9	92	22.0	13.8
		Pythium	3	8	80	8.0	4.0
		Helminthosporium	3	6	100	20.0	12.2
27 to 29	60	Control	2	5	100	22.0	19.0
		Sterile medium	3	4	92	22.0	17.5
		Fusarium	3	5	98	22.0	13.4
		Pythium	3	6	96	13.0	6.6
		Helminthosporium	3	6	100	17.0	13.9
	90	Control	2	4	100	28.0	37.5
		Sterile medium	3	7	100	24.0	20.0
		Fusarium	2	8	94	21.0	18.6
		Pythium	3	6	92	13.0	7.2
		Helminthosporium	3	5	100	20.0	19.1

* *W.H.C. = water holding capacity.*

The Effect of Controlled Soil Temperature and Moisture on the Action of the Root Rot Pathogens

In the following soil temperature and moisture studies, steamed compost in controlled temperature tanks (14) was inoculated with species of *Pythium*, *Helminthosporium*, and *Fusarium*, isolated from corn roots, to determine the specific effect of the three types of organisms on corn growing under constant controlled soil conditions. Temperature ranges of 9° to 11° C., 13° to 15° C., 17° to 19° C., 22° to 24° C., and 27° to 29° C. were used and soil in duplicate containers at each of the temperatures was kept respectively at approximately 60 to 90% of its water holding capacity. Cultures of two species of each of the genera of pathogens were mixed with the surface four inches of soil in single containers and checked against uninoculated steamed compost and similar compost inoculated with the same sterile medium as that used in the culture of fungi. Each container was then planted with corn (Fig. 15).

The results in Table III show that at all but the lowest temperature (9° to 11° C.) high moisture increased the growth perceptibly over the corresponding series kept at the lower moisture content. Where no pathogen was involved, the percentage germination was not affected by either temperature or moisture, but its rapidity was reduced in direct proportion to the soil temperature. A distinct chlorosis was present in the plants growing at the lowest temperature (9° to 11° C.) with 90% moisture, a condition not present at any of the other temperatures.

The addition of sterilized culture medium to the soil appreciably reduced the growth of corn. This reduction was most pronounced in the moist soils and at the higher temperatures, being proportionately less in the lower temperature ranges.

Pythium spp. proved to be the most strongly pathogenic of the three genera tested, the effect on the host being reflected in extreme reduction in growth even at the highest temperature, which is the optimum for the development of the host (4). There was also an indication of pre-emergence killing of the seedlings evident even at the highest temperature range. This was more pronounced in the moister soils and was increasingly severe with the lowering

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FIG. 12. *Corn growing in root rot soil following four cover crops of (1) wheat, (2) oats, (3) rye, (4) timothy, (5) soybeans, (6) red clover.*

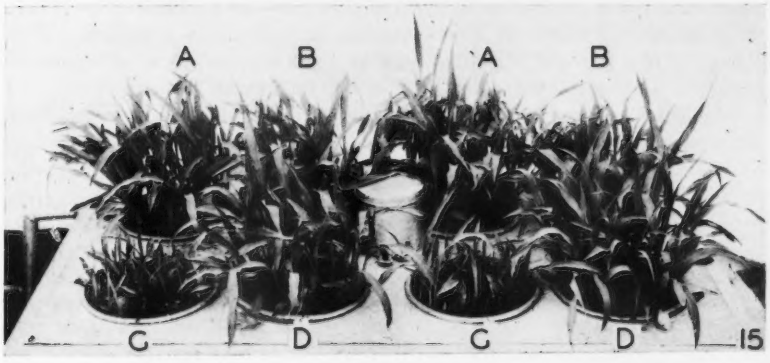
FIG. 13. *Same as Fig. 12, following (1) alfalfa, (2) rape, (3) buckwheat, (4) sugar beets, (5) tomatoes, (6) corn.*

FIG. 14. *Corn growing on root rot soil following crop rotations of:—*
(1) buckwheat, red clover, timothy, wheat;
(2) oats, soybeans, rape, alfalfa;
(3) soybeans, wheat, alfalfa, sugar beets;
(4) red clover, buckwheat, oats, timothy;
(5) rape, rye, timothy, soybeans.

FIG. 15. *Corn 14 days after planting at constant soil temperature of 22° to 24° C., not inoculated, A; inoculated with Fusarium spp., B; Pythium spp., C; and Helminthosporium spp., D. The four containers at left were kept at 60% water holding capacity, those at right. 90%.*

of the temperature range. Many of the leaves also showed considerable malformation and failed to unfold.

The general effect of inoculum of *Helminthosporium* spp. on the subsequent crop of corn was similar to, though less severe than that of *Pythium* spp.



There was no appreciable pre-emergence killing of the seedlings except at the two lowest temperature ranges.

The detrimental effect on the corn growing in soil inoculated with *Fusarium* spp. was much less marked than with either of the other fungi. Regardless of temperature or moisture there was no reduction in germination and only a slight reduction in growth.

In each series the leaf growth coincided closely with the root development, the amount of parasitism being definitely reflected in the vigour of the plants. The particular type of injury, however, varied with the parasite involved. In roots invaded by *Pythium* infection usually commenced at the apex of the root and progressed rapidly as a light coloured, watery soft rot, destroying first the cortical tissues and later the vascular system. All roots, regardless of size, appeared subject to infection, but unless the seed was destroyed, the mesocotyl was seldom affected (Figs. 7 and 8). Roots infected by *Helminthosporium* spp. were dark brown or black and leathery in consistency. The scutellum and endosperm as well as the mesocotyl were usually infected as were most of the radicles and crown roots. The laterals on the infected areas seldom survived, but owing to the fact that the stele was not destroyed, the roots beyond the infected areas apparently functioned quite normally and sustained plant growth (Fig. 10). Quite frequently the infection travelled upwards and involved the coleoptile and older leaves. *Fusarium* spp. had a definite, though considerably less pronounced, parasitic effect resulting in the destruction of many laterals and occasionally causing light brown lesions on the larger roots (Fig. 11). In contrast with the injury caused by *Pythium* spp. and *Helminthosporium* spp., that induced by *Fusarium* spp. was more severe at the higher temperatures where the soil moisture content was low, and was practically negligible at the lower ranges.

With the exception of a few isolated contaminations it was found that the pathogens present in the corn roots were those that had been incorporated with the soil as inoculum.

Discussion

Regarding the organisms mentioned in this paper as being associated with root rot of corn, *Pythium arrhenomanes* has been described elsewhere (6, 18, 20) as a strong parasite affecting sugar-cane as well as corn. *Pythium de Baryanum*, although long considered a soil inhabiting parasite and held responsible for severe infections of many plants, has just recently been associated with root rot of corn by Wen-Chun Ho and Melhus (8), Melhus *et al.* (17), and Wen-Chun Ho (7). These investigators also mention having isolated *Helminthosporium sativum* and *Fusarium* spp. from roots of corn, but consider them of secondary importance. The observations made during the progress of the present investigation, however, leave little doubt that in addition to *P. arrhenomanes*, members of the genus *Helminthosporium* and to a lesser degree *P. de Baryanum* may be important factors in the corn root rot problem in Ontario.

Although organisms other than those dealt with in this paper may have a bearing on the corn root rot problem, the foregoing experiments prove that the organisms herein considered have definite parasitic capabilities and are the ones most frequently associated with the disease in Ontario. This, coupled with the fact that sterilized soil produces root rot free plants, suggests that regardless of the other conditions that may be involved, parasitic organisms play an important role in root rot of corn.

Since typical root rot symptoms have been produced in corn growing on different types of soil from various localities, it seems likely that with the advent of favourable environmental conditions the disease might be of significant economic importance in these areas.

While microscopic examinations of the roots of many species of plants growing in infected soil reveal a similarity in the types of organisms invading the tissues, there is a definite variation in the amount of infection and extent of root injury produced. Whether the growth of certain crops alters the balance of micro-organisms in the soil and reduces the quantity of parasitic forms present, or causes some chemical change in the soil unfavourable to the development of certain harmful organisms, the fact remains that in root rot soil in the greenhouse, corn following some crops in a rotation, notably soybeans, is practically free of root rot.

There is little doubt that soil temperature and moisture have a direct bearing on the corn root rot problem, since cool moist conditions which retard the germination of corn seed are favourable for the development of several strong root rot parasites.

Acknowledgments

Sincere thanks and appreciation are tendered Dr. G. H. Berkeley for advice and criticism during the progress of this investigation, and to Drs. J. E. Machacek and T. C. Vanterpool for their identification of fungi.

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THE STRUCTURE AND DEVELOPMENT OF THE CHROMOSOME SPIRALS IN MICROSPORES OF *TRILLIUM*[1]

By A. H. Sparrow[2]

Abstract

The structure and development of somatic spirals in microspore chromosomes of *Trillium* have been investigated. The chromonemata in each metaphase chromatid and each anaphase chromosome form a large-gyred, hollow spiral. This spiral develops gradually during prophase by an increase in gyre diameter and a decrease in gyre number and in chromatid length. Its development is associated with the *elimination* not the *production* of chromatid relational coiling. At later stages an irregular waviness or "minor" somatic spiral is visible along its "major" gyres in which reversals of direction can also be discerned. Where the spiral can be seen to be double-stranded it is plectonemic (as early as mid-prophase).

The prophase to metaphase chromatid contraction ratio is not less than 6 : 1. Mean chromonema length increases from 650 ± 17.2 at metaphase to 977 ± 28.3 at anaphase. This latter length is approximately that estimated for early meiotic prophase. Chromosome volume also increases (about twofold) during the interval between metaphase and anaphase. Mean chromonema length and gyre number in microspore anaphase chromosomes are more than twice as great as those of meiotic anaphase chromosomes. Since the chromosomes at these stages are of approximately the same mean length the gyres of the somatic spiral are thus more tightly "packed".

In *Trillium*, microspore anaphase chromosomes are considered to be of essentially the same spiral structure as meiotic second division chromosomes, i.e., a single coil (but *not* single-stranded), rather than two or more independently coiled chromatids. The process of reducing this plectonemic spiral into parallel, freely-separable chromatids begins in one prophase as a reduction in gyre number and continues as relational uncoiling in the next. Paradoxically, therefore, a spiralization cycle such as that described above can be interpreted as an uncoiling process in which successive cycles overlap in prophase.

Introduction

As a prerequisite to any study of chromosome mechanics a thorough grasp of their micromorphological features throughout all stages of the mitotic cycle is of paramount importance. Unfortunately, previous investigators have differed widely in their interpretations of somatic chromosome structure and naturally those who have considered the mechanics of spiralization have formulated theories almost as diverse as their interpretations of structure.

It is now generally recognized by cytologists that the chromonemata of somatic chromosomes are spiralized to a variable degree at all stages of mitosis (e.g., 2). The disagreement arises as to the number of spirals, their structure, interrelationships, and ontogeny. Different teleological interpretations are also given. The present investigation of mitotic chromosomes of *Trillium* was initiated in the hope of clarifying at least some of these important problems, with the ultimate goal of a single unified hypothesis to explain both somatic and meiotic coiling.

[1] *Manuscript received September 22, 1942.*
Contribution from the Department of Genetics, McGill University, Montreal, Que.

[2] *Holder of a Fellowship under the National Research Council of Canada.*

A clear understanding of the spiral structure of somatic chromosomes has lagged considerably behind the elucidation of meiotic chromosome structure. There is no dearth in the early literature of descriptions or illustrations of telophase and prophase spirals, but the relatively rare observations of metaphase or anaphase spirals led to the conclusion that the appearance of spiral structure was the exception rather than the rule (44). It is now apparent that one of the most important factors responsible for this unfortunate situation is that techniques which successfully demonstrate spiral structure in meiotic chromosomes usually show relatively little spiral structure at somatic metaphase and anaphase. The greater chromaticity of the matrix, as well as the closer packing of the gyres of somatic chromosomes, is, presumably, involved in determining this characteristic difference. Much confusion has also arisen from observations of chromosomes showing a high degree of vacuolation. Such a structure is now generally considered to be an artifact resulting from poor fixation (14, 20, 31).

The spiral structure of mitotic anaphase chromosomes or metaphase chromatids has been variously interpreted by different investigators to fall within one of the following categories: (i) a single spiral, (ii) two non-intertwining threads, (iii) two intertwining threads, or, (iv) two or more independently spiralized threads that may be more or less twisted about each other[1]. According to some interpretations these categories overlap somewhat, as in the case of early anaphase chromatids being parallel but becoming progressively twisted about each other at later stages (1). Also it is considered by some investigators that an apparently single-stranded spiral may actually be composed of two or more closely appressed chromonemata that may not appear visibly distinct until telophase or the following prophase (58, 18, 20, 53, 1, 43, 7, 8). The rather contradictory views in the literature on the number of chromonemata Kuwada (38) explains "by the assumption that under certain circumstances the four chromonemata appear by union to be two or even one." This may account for contrasting reports of double-stranded vs. single-stranded spirals, but it is rather difficult to imagine four, or even two, independently spiralized chromonemata suddenly merging into one optically single-stranded spiral.

In accord with the greater attention paid to meiotic than somatic spirals, theories of spiralization have been based largely on such studies. The various theories so far formulated may be roughly divided into two groups, those in

[1] *The investigators upon whose work this classification is based include the following: (i) Kuwada, 1926 (36); Geiller, 1935 (18), 1938 (19, 20); Sax and Sax, 1935 (53); Gustafsson, 1936 (22); Upcott, 1936 (64); Darlington, 1937 (13); Mather, 1937 (42); While, 1937 (67); Darlington and Upcott, 1939 (15), 1941 (16); Manton, 1939 (40); Coleman, 1940 (7, 8); Hillary, 1940 (24); (ii) Sharp, 1929 (57); Straub, 1938 (61); (iii) Brunelli, 1910 (6); Schneider, 1910 (56); Dehorne, 1911 (17); Kaufmann, 1926 (31, 32); Telezynski, 1930 (62), 1931 (63); Hedayetullah, 1931 (23); Hsu-Siang, 1932 (26); Perry, 1932 (52); Smith, 1932 (58); Koshy, 1933 (34), 1937 (35); Hoare, 1934 (25); Marshak, 1936 (41); Jeffrey, 1937 (30); Naithani, 1937 (45, 46); Atwood, 1937 (4); Warmke, 1937 (66); Wolcott, 1937 (69); Creighton, 1938 (9); Mensinkai, 1939 (43); (iv) Nebel, 1933 (47); Goodspeed, Uber, and Avery, 1935 (21); Stebbins, 1935 (60); Nebel and Ruttle, 1936 (50); Kuwada, 1939 (38); Kuwada and Nakamura, 1940 (39); Aisima, 1941 (2).*

which the matrix is regarded as the dynamic force with the chromonema playing a minor role (3, 54, 55) and those in which changes within the chromonema are of paramount importance (36, 37, 5, 11, 27, 48, 68). These theories cannot be discussed in detail here. Suffice it to say that they include such mechanisms as contraction of the matrix, elongation of the chromonema, anisotropic swelling, molecular reorientation, heterogonic growth, pH changes, etc. Obviously many of these postulated mechanisms cannot be tested experimentally, but two factors are now known definitely to be associated with the formation of the major coil of meiosis. They are shortening of the matrix (in *Tradescantia*) and elongation of the chromonema (in *Trillium*). In mitosis it is known that the development of the new somatic spiral in each prophase is associated with chromatid (hence matrix?) contraction, but so far no one has measured chromonema length changes during these stages.

Somatic spirals can be seen to increase in diameter from early prophase to metaphase and still further in their expansion into relic coils. However, it is not agreed whether or not the gyre number decreases as their size increases. Husted (29), Koller (33), and Abraham (1) consider that they do decrease in number, while Nebel (49) holds the opinion that the prophase gyres are "not much, if any, more numerous than the final standard gyres."

Materials and Methods

Observations were made on microspore chromosomes of two species of *Trillium*. The data presented herein are from *T. grandiflorum*. Less extensive observations have also been made on *T. erectum*. The best preparations for internal structure were smears stained in iron-aceto-carmine. After replacement with 45% acetic acid and sealing, the slides were kept in a refrigerator until used. The internal structure seemed clearer in old slides than in material freshly prepared. Some of these "temporary" slides are still in good condition more than a year after their preparation.

The methods used in making measurements have been described by Sparrow, Huskins, and Wilson (59) and will not be repeated in detail here, except to give the formula used in calculating the length (L) of a spiralled thread:

$$L = n\sqrt{p^2 + (\pi d)^2}$$

when n = number of gyres, p = pitch, and d = diameter of the gyre minus the diameter of the chromonema. All lengths given are totals for a set of five chromosomes.

Observations

Morphologically, chromatid contraction is the most obvious change that chromosomes undergo during prophase. This contraction is accompanied by two processes which at first sight seem antithetic: (1) the unwinding of the (relic) spirals carried over from the previous anaphase, and (2) the development of a new somatic spiral within each chromatid. In a previous paper the unwinding of the relic coil of meiosis and the origin and elimination of chromatid relational coiling in the microspore prophase have been considered (59).

The study has now been extended to include the accompanying structural changes within the chromatids, i.e., the structure and development of the somatic spiral from early prophase through metaphase and anaphase of the first division of the microspore. Two illustrations of spiral structure at prophase of the following division (i.e. pollen tube) are also presented.

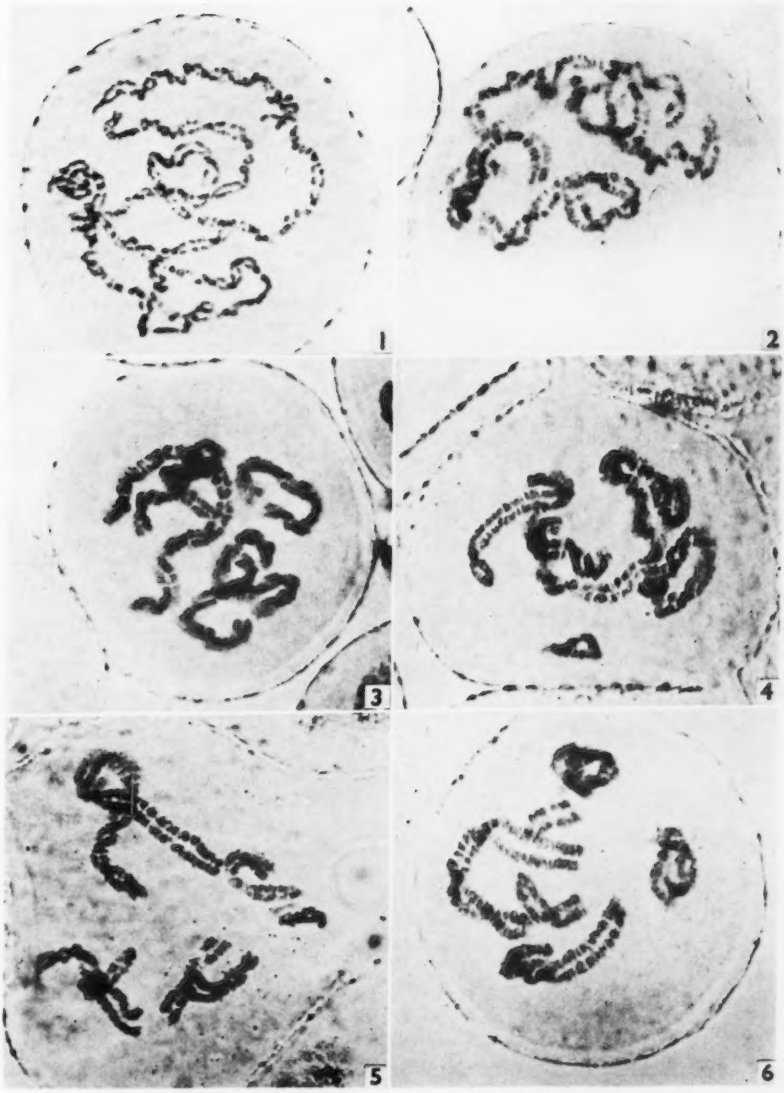
Chromatid lengths in 10 microspore prophase nuclei varied between 410 and 590 μ, with a mean of 467 $\pm$ 17 μ. The high variation is, presumably, due to the fact that prophase contraction has proceeded further before fixation in some nuclei than in others. The above mean is therefore regarded as an underestimate of early prophase length. A length of at least 500 μ would be a conservative estimate of the initial length, 600 μ would not be unexpected. Mean metaphase chromatid length measured in 15 cells is 90.5 $\pm$ 3.9. These figures give a contraction ratio of about 6 : 1 between early prophase and metaphase. No significant change occurs in chromatid length between metaphase and anaphase, but mean chromatid diameter increases significantly. The total increase in chromatid diameter between early prophase and anaphase is more than threefold (from less than one to almost three micra).

The relic spirals of microspore prophase are derived from the meiotic major spiral. Likewise it seems that the waviness visible along the gyres of the relic coil corresponds to the "minor spiral" of meiosis. This has been checked by counting the number of "waves" and "minor gyres" at the two stages. Their number is estimated to be between 480 to 600 for a coil of 60 gyres, i.e., about 8 to 10 per gyre of the major or relic coil. During early prophase the irregularly waved chromonemata within the chromatids gradually assume the form of a regular spiral. The growth of this spiral by an increase in gyre diameter and a reduction in gyre number is illustrated in Figs. 1 to 8. Chromatid lengths and gyre number at various stages from early prophase to anaphase are given in Table I. As the number of gyres in prophase stages cannot readily be determined by direct counting throughout whole complements the total number of gyres per complement has been estimated from counts made

TABLE I

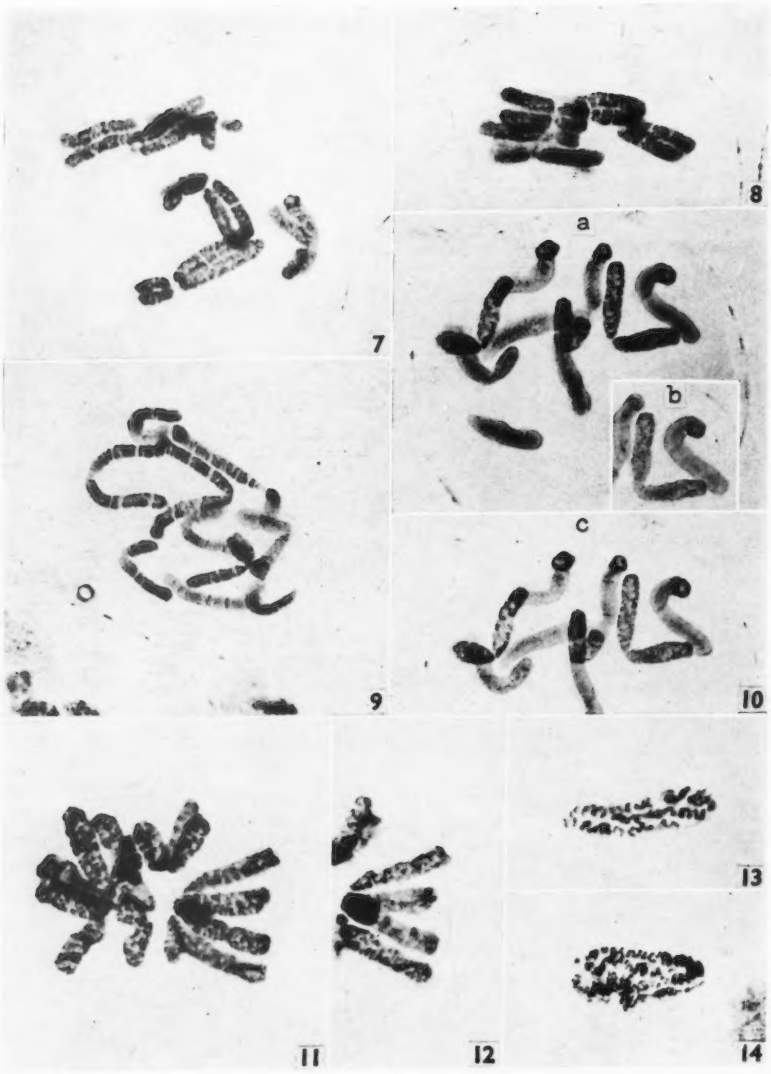
CHROMATID LENGTH (IN MICRA) AND GYRE NUMBER AT PROPHASE, METAPHASE, AND ANAPHASE STAGES IN MICROSPORES OF *T. grandiflorum*

Stage	Chromatid length	No. of gyres
Relic coil of 60 gyres	450–600	480–600
Prophase (Fig. 1)	346	554
Mid-prophase (Fig. 2)	202	242
Mid-prophase (Fig. 3)	205	276
Later prophase (Fig. 4)	173	151
Later prophase (Fig. 5)	154	170
Later prophase (Fig. 6)	142	187
Metaphase (Fig. 8)	77	130
Metaphase (Mean of 15 cells)	90.5 $\pm$ 3.9	120 $\pm$ 5.1
Anaphase (Mean for 15 cells)	95.0 $\pm$ 2.9	130 $\pm$ 3.3



Prophase stages showing progressive changes in somatic and relational coiling in microspores of T. grandiflorum. Magnification, 1000×. From temporary aceto-carmine smears.

FIG. 1. *Prophase (346 μ and 554 gyres).* FIGS. 2 AND 3. *Mid-prophase (202, 205 μ and 242, 276 gyres, respectively).* FIGS. 4, 5, AND 6. *Later prophase stages (173, 154, 142 μ and 151, 170, and 187 gyres, respectively).*



Spiral structure in metaphase and anaphase of microspore mitoses and relic coils in pollen tube prophase nuclei. FIGS. 7 TO 12, *T. grandiflorum (a and c of Fig. 10 are different prints made from the same negative, b is taken at a slightly different focus);* FIGS. 13 AND 14, *T. erectum. Magnification, 1000×. From temporary aceto-carmine smears.*

FIGS. 7 AND 8. *Metaphases.* FIGS. 9, 10, 11, AND 12. *Anaphases. In Figs. 7 and 10 the optical cross sections show the irregularly waved chromonemata and the hollow spiral (See text, p. 261).* FIGS. 13 AND 14. *Relic coils in pollen tube prophase nuclei of T. erectum.*

in regions where the gyres are most clearly visible. Lengths in these regions were obtained from measurements of camera lucida drawings which were frequently checked from photographs or with micrometers.

Measurements of metaphase and anaphase chromonema lengths have been made in five cells at each stage in three different plants (Table II). The mean gyre diameter, gyre length, and total chromonema length all increase significantly. Mean chromonema length for 15 cells increases from 650 ± 17.2 at metaphase to 977 ± 28.3 at anaphase. The latter length is approximately that of the early meiotic prophase of *T. erectum* (59). Chromosome volumes have not been given in the table but calculations made from the data presented indicate that the mean volume at anaphase is about double that at metaphase.

TABLE II

MEASUREMENTS AT METAPHASE AND ANAPHASE OF SOMATIC SPIRALS IN *T. grandiflorum* MICROSPORE COMPLEMENTS

Plant number	No. of cells	Stage	Gyre diam.	Chromosome length	No. of gyres	Chromonema length	Mean gyre length
69-*G*-92-*G*	5	*M*	2.34±0.245	100±7.7	104±3.1	602±46.8	6.57±0.45
	5	*A*	3.10±0.185	101±2.9	118±6.6	1002±27.9	8.82±0.53
69-*G*-100-*G*	5	*M*	1.95±0.131	83±4.7	136±11.3	682±6.1	5.16±0.44
	5	*A*	2.54±0.072	89±7.0	137±4.0	917±27.3	6.66±0.26
69-*G*-104-*A*	5	*M*	1.98±0.087	88±5.2	121±1.9	644±21.5	5.32±0.27
	5	*A*	2.68±0.133	94±3.8	134±2.4	1011±39.9	7.53±0.42
69-*G*-92,-100, and -104	15	*M*	1.95±0.097	90.5±3.9	120±5.1	650±17.2	5.68±0.27
	15	*A*	2.77±0.099	95±2.9	130±3.3	977±28.3	7.67±0.30

Metaphase and anaphase microspore chromosomes have essentially the same structure as those of the second meiotic division, i.e., a single double-stranded spiral. The gyres are, however, more numerous, closer together, and hence more difficult to distinguish. The somatic spiral is composed of two visibly distinct chromonemata, which, like the half-chromatids of meiosis, are irregularly waved. This waviness can be seen in optical cross sections (Figs. 7, 10) as well as in side views (Fig. 11) and in neither case does it approach the form of a regular spiral. In certain selected regions the two chromonemata can be seen to be wound in the form of a plectonemic spiral even as early as mid-prophase. There seems to be considerable variation as to the closeness of the two associated chromonemata. In some regions they are quite widely separated and in others even within the same chromosome they appear as a single-stranded spiral. Changes of direction have also been observed but their frequency has not been determined.

The chromonemata of metaphase and anaphase chromosomes occupy only the peripheral region of the chromosome. There is a central core of lower chromaticity. This is best seen in optical cross sections (Figs. 7, 10), but can

also be seen in longitudinal optical sections (Fig. 7, right arm of lower chromosome). The latter is considered by the author to explain the frequent claims in the literature that anaphase chromosomes are composed of two independently spiralized chromatids (2). With a slight change of focus these so-called "independently spiralized chromatids" mysteriously disappear to give cross-striations extending right across the chromosome (Figs. 8, 9). The latter represent the true gyres. The former are optical illusions.

Preliminary observations of pollen tube mitoses have been made in regard to chromosome structure and mechanics. The results obtained essentially confirm those obtained from studies of microspore mitosis. Here again, relational coiling is derived from plectonemic relic coils (Figs. 13, 14) and the somatic spirals apparently "grow up" by an increase in gyre diameter and a decrease in number.

Observations have also been made on meiotic spirals in *Tradescantia* by Dr. S. G. Smith. He considers that the minor spiral present at first anaphase (which is a more regular spiral than the corresponding waviness in *Trillium*) develops into the typical second anaphase spirals. The major coil of first division is eliminated at the prophase of the second division in *Tradescantia* while it persists in *Trillium* until microspore prophase. In the latter case the second division occurs without any intervening interkinetic resting stage and is not accompanied by a new spiralization. This is of especial interest in that it shows that spiralization does not always keep pace with the spindle mechanism. In the absence of an interkinetic resting stage in *Trillium* the new spiralization does not occur until microspore prophase, but the activity of the spindle is, to all appearances, normal. A comparative physiological study of meiosis in species with and without interkineses might throw some light on the changes determining nuclear or chromosomal transformations as distinct from those associated with spindle activity.

Discussion

The value to theoretical cytology of accurate measurements of such obviously important factors in the chromosome spiralization cycle as size changes cannot be overestimated. Unfortunately this has been recognized by only a few cytologists, e.g., Wilson and Huskins (68), Manton (40), and Darlington and Upcott (15). Meiotic changes especially at prophase have been studied by a number of workers (see summary, by Darlington and Upcott, 15) but mitotic stages have been almost neglected. Prophase chromatid contraction has been measured by Sax and Sax (53) and by Pätau (51). The former found a prophase to metaphase ratio of 5 : 1 in *Tradescantia* microspores and the latter, 5.5 : 1 in *Collozoum*. These ratios agree very closely with the 6 : 1 ratio for *Trillium* microspores reported here.

As far as the author is aware, Manton (40) is the only worker who has measured lengths of spiralled chromonemata in mitotic chromosomes. From her work on *Osmunda* she states that "the real length of the chromosome thread is . . . . . thought to be constant throughout the mitotic cycle."

Bridges (3), and Naithani (46) are also of the opinion that the gene thread or chromonema maintains a constant length. The present data which show an elongation of the chromonema between metaphase and anaphase in microspore chromosomes of *Trillium* are not in accord with the opinions of the above workers, for an increase as great as 327 μ (more than nine times the standard error) can hardly be due to chance variations either in absolute lengths or in errors of measurements. Nor can the increase be attributed to the straightening out of a "minor spiral" for the irregular waviness at anaphase appears more copious than at metaphase.

Darlington and Upcott (15) have abstracted some of the published work on chromosome and chromonema size. They find that the measurements of "several workers" agree in giving what they call a "packing factor $\frac{D}{d}$ of 2.0 to 2.5". ("D = diameter of chromatid, d = diameter of chromosome thread or of the region occupied by it".) As "probably most compatible with observation and with the model" their illustrations of chromosomes representing this value are solid spirals. The observations on *Trillium* spirals are not in accord with this conclusion as both meiotic major and somatic spirals are clearly seen to be hollow. Chromosomes in fresh pollen mother cells of *Trillium* mounted in 4% cane sugar solution also show a hollow spiral structure according to Wilson (unpublished data).

It has been stated above that the somatic spiral is considered to grow up by an increase in gyre size and decrease in gyre number. In tests with models the expanding gyres proximal to the attachment get their extra length at the expense of the distal ones. Since gyre diameter seems to be fairly uniform for a chromosome arm, at a given stage, but increases gradually over a period of time the expansion per gyre will be proportional to the number lost at the end. Hence each time a gyre is lost the redistribution must extend all down the line. Redistribution can, however, occur from changes of direction as well as from the end. It seems probable, therefore, that reversals may play a part in the development of the somatic spiral as well as in untwisting of prophase chromatid relational coiling as previously described (59).

It is the opinion of the writer that the pairs of chromonemata separated by the quaternary split (the split that becomes effective at anaphase of pollen tube mitosis) are plectonemic at mid-prophase of the first microspore mitosis. They may also be plectonemic (or relationally coiled) at earlier prophase stages before their relationships can be determined by optical methods. In this connection it should be borne in mind that the time at which the plane of the split is determined is at least *as* important, if not more important, than the time at which it becomes visible. Obviously information on the former point is beyond hope of attainment with present techniques, and one can do no more than speculate as to how and when the plectonemic condition arises. If it were to arise after the initiation of the somatic spiral the split would necessarily be a spiral cleavage in the same direction as the gyres of the spiral. If the plane were already present or determined before the chromonemata

became regularly coiled the attainment of the plectonemic condition might require that the chromonemata twist about each other—or they might untwist, this depending upon the original amount of twisting present.

Several authors have suggested that in mitosis the daughter chromatids are twisted about each other at the time of their inception (31, 35, 46, 38). This seems not unreasonable and is in accord with their observed plectonemic condition at mid-prophase in *Trillium* microspores. Before the sister threads in this plectonemic coil can separate at the anaphase of the following division they must undergo extensive unwinding. Further, the amount of unwinding required is proportional to the number of gyres present. Therefore any reduction in gyre number of a plectonemic coil is the equivalent of untwisting in the derived relational coil. Hence the untwisting or unwinding process necessitated by the fact that the somatic coil is plectonemic begins with the decrease in gyre number in one prophase, continues in the further reduction during the transition to the relic coil of the next prophase and is completed with the final elimination of the twists in the chromatid relational coil derived from the plectonemic relic.

In the above interpretation prophase chromatid untwisting is a continuation of the changes initiated in the previous division and not a reversal (*contra* Abraham, 1). Concurrent with this untwisting a new spiral is developing within each chromatid so that successive cycles overlap in prophase. But, paradoxically, even the further development of this new spiral is an unwinding process in that any reduction of gyres is the equivalent of an elimination of relational twisting. In the material studied there has been found no sudden reversal of the process of untwisting to one of retwisting (cf. 38, 1) nor any evidence of chromatids actively twisting about each other during prophase as frequently suggested (12, 28, 65, 10). The growth of the new internal spiral seems to be associated with the *elimination* of relational twisting and not with its *production*, as suggested by some of the authors referred to above. Further, if a process of active twisting is to be postulated to account for the plectonemic condition, it must occur within each chromatid one division in advance of that at which it is finally undone and at a very early stage before the fine threads can be observed with sufficient resolution to detect it. The amount of twisting necessary on this hypothesis to give the observed number of plectonemic gyres would seem to be prohibitive. It is therefore tentatively suggested that the twisted condition that presumably prefaces plectonemic coiling may be structurally inherent rather than the result of torsion affecting the pairs of chromonemata or chromatids after their dual nature can be optically resolved.

Acknowledgments

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CHROMOSOME BEHAVIOUR AND FERTILITY IN DIPLOID WHEAT WITH TRANSLOCATION COMPLEXES OF FOUR AND SIX CHROMOSOMES[1]

By W. P. Thompson[2] and Isabel Hutcheson[3]

Abstract

Seven different reciprocal chromosome translocations involving six of the seven haploid chromosomes of *Triticum monococcum* have been studied in all possible heterozygous combinations. Plants with one complex of four chromosomes show only 5 to 10% sterility; those with two complexes of four, 10 to 20%; those with one complex of six, 20 to 30%. Completely random segregation in such types should cause 66.6, 88.8, and 90% sterility, respectively, while segregation directed only by the necessity that homologous centromeres go to opposite poles should cause 50, 75, and 75%, respectively. The very low sterility of translocation heterozygotes in wheat, as compared with these expectations, and with results reported in maize and other plants, is due to the fact that segregation is usually alternate (disjunctional) in complexes both of four and six.

The forces of repulsion operating at metaphase are not restricted to the centromere but involve the whole body of the chromosome. In the absence of complicating features this naturally results in alternate segregation in complexes. Semisterility is due, not to basically random segregation, but to special conditions such as interstitial chiasmata, early opening of the complex, and crossing over between the centromere and the point of interchange. The latter is favoured by a non-median position of the centromere, great length of chromosomes, and shortness of at least one interchanged segment. The 50% sterility usually reported for translocations has no special significance; no particular percentage is characteristic of translocations in general.

In complexes of six, double-cross configurations are more numerous than stars, and present many variations in form depending on the length and position of the segments exchanged. Additional factors producing sterility in large complexes are unwieldiness and crossing over in the segment that joins the two crosses.

Introduction

Plants that are heterozygous for reciprocal chromosome translocations show, in different cases, very different degrees of sterility. The impression that as a rule they are semisterile is apparently due to the fact that much of the work on translocations, and particularly the early work, has been carried on with maize, in which nearly all are reported to involve sterility of 50%. In fact, maize plants that are heterozygous for a translocation are commonly referred to as "semisteriles". The situation in peas is similar. But several examples in wheat were shown to be almost fully fertile (13, 14). Nearly complete fertility appears to be the rule also in *Datura*. In other genera, such as *Tradescantia* and *Campanula*, although sterility has been reported, it may be much less than 50%.

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The causes of these differences in sterility must be bound up with the causes of the sterility itself. The reason always given for the situation in maize is that random two-by-two segregation takes place in the translocation complex of four chromosomes. When any two chromosomes that are adjacent in the ring go to the same pole the resulting spore always has both a deficiency and a duplication and is therefore inviable (Fig. 1, F to I), whereas when alternate chromosomes go together (Fig. 1, D and E) the resulting spore always has the normal chromatin complement (either in the original or in the new arrangement) and is therefore fully viable. It has been assumed that "alternate" and "adjacent" segregations are equally frequent and hence that the sterility is 50%. But in true random segregation two-thirds of the spores (not one-half) receive adjacent chromosomes, as is demonstrated in Fig. 1. Consequently random segregation should result in 66% sterility, not the reported 50%.

The percentage of incompetent spores would be reduced from 66 to the observed 50% if two chromosomes whose centromeres are paired in prophase (homologous) must always go to opposite poles. In that case the two combinations *F* and *I* of Fig. 1 would not be formed. But since the complex opens out into a ring before segregation there is no apparent reason why homologous centromeres should go to opposite poles. Furthermore McClintock (9) has shown that in maize they actually may go to the same pole.

The reason that fertility is nearly complete in the wheat plants studied is that segregation from the complex of four chromosomes is regularly alternate, according to Thompson and Thompson (14). At metaphase the complex regularly takes the form of a horizontal figure of eight. Smith (12, 13) did not observe this directed segregation and was therefore at a loss to account for the high fertility. There can be no question that, in the material studied in the present investigation, the segregation is alternate in the great majority of cases; the amount of adjacent segregation corresponds closely to the amount of sterility.

The problem therefore is to determine why segregation should be regularly alternate (disjunctional) in one genus and random in another. On general principles one would expect that the forces that cause the segregation of the chromosomes would work out in the same way in all plants. It was therefore decided to undertake the following studies: (1) an investigation of additional translocations in wheat and of hybrid combination with two complexes of four chromosomes, in order to leave no doubt as to whether disjunctional segregation, resulting in full fertility, must be regarded as the normal procedure in wheat; (2) a careful study of chromosome behaviour, particularly during prophase, in comparison with other genera, with a view to determining the reasons for the differing behaviour.

A similar set of problems arises in connection with complexes of six chromosomes. Such a complex is produced when a cross is made of two types each

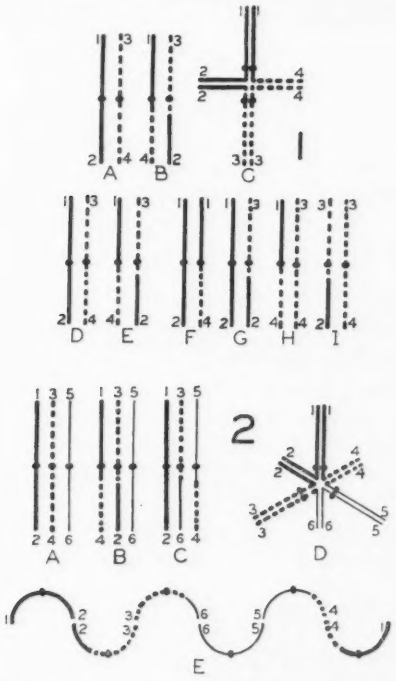


FIG. 1. *A, original chromosomes; B, after reciprocal translocation; C, cross-shaped complex in meiotic prophase; D and E, chromosomes resulting from alternate segregation; F to I, chromosomes resulting from adjacent segregation.*

FIG. 2. *A, original chromosomes; B, after first translocation; C, after second translocation; D, star-shaped complex at prophase; E, zigzag configuration at metaphase in alternate segregation (each set of three chromosomes (going up or down) includes a full complement of chromosome material, and would result in a fully viable spore).*

of which is homozygous for a different translocation involving a common chromosome. In maize such plants are reported to show 70% sterility. If segregation from the complex were always alternate only two combinations of chromosomes would be produced, as shown in Fig. 2, and both of these would be viable; consequently no sterility should occur. But if segregation is random three-by-three, 20 different combinations would occur with equal frequency. The only viable combinations would be the two that result from alternate segregation. Therefore random segregation would cause 90% sterility instead of the 70% reported for maize. If segregation were such that homologous centromeres must always go to opposite poles but is not otherwise directed, eight combinations would be formed with equal frequency, as may be seen by reference to Fig. 2. Again only two would be competent; consequently the sterility should be 75%. It was therefore decided to investigate complexes of six in the same way as complexes of four.

The Translocations and Their Relationships

Seven reciprocal translocations in *Triticum monococcum*, produced by radiation treatment, were used. These were designated T_1, T_2, ... T_7. Homozygous types, which are all fully fertile and indistinguishable from the original material, were crossed in all possible combinations, and all F_1 combinations were examined cytologically to determine whether they had two complexes of four chromosomes or one of six. The results are given in Table I. From the data in this table the relationships of the chromosomes involved in the different translocations may be worked out.

TABLE I

CHROMOSOME COMPLEXES IN HYBRIDS BETWEEN LINES HOMOZYGOUS FOR DIFFERENT TRANSLOCATIONS. TWO COMPLEXES OF FOUR CHROMOSOMES ARE DESIGNATED "4", AND ONE OF SIX IS DESIGNATED "6"

Translocation number	Translocation number						
	1	2	3	4	5	6	7
1	x	6	6	6	4	4	4
2		x	4	6	4	4	6
3			x	6	6	4	6
4				x	4	4	6
5					x	6	6
6						x	4
7							x

Let the original seven haploid chromosomes of *T. monococcum* be designated 1.2, 3.4, ... 13.14, each pair of numbers representing the two ends of a single chromosome. Let the chromosomes involved in T_1 be 1.2 and 3.4, and let the chromosomes with successively higher numbers be assigned to successively higher-numbered translocations, in so far as this is necessary to satisfy the data of Table I. The results are given in Table II. The conclusions shown in this table are throughout consistent with the data given in Table I, and are necessary in order to account for those data. Observations

TABLE II

THE CHROMOSOMES INVOLVED IN EACH OF THE TRANSLOCATIONS STUDIED

Translocations	Chromosomes						
	1.2	3.4	5.6	7.8	9.10	11.12	13.14
T_1	1.2	3.4					
T_2		3.4	5.6				
T_3	1.2			7.8			
T_4	1.2		5.6				
T_5				7.8	9.10		
T_6					9.10	11.12	
T_7			5.6	7.8			

to be reported later show that when a particular chromosome was involved in more than one translocation the segments transferred came either from the same or from the two different arms.

It will be observed that with one exception (13.14) each of the seven original chromosomes is involved in at least one translocation. Three of them are involved in three different translocations, two in two, and one in one. The study of these seven should, therefore, give a representative picture of the behaviour of translocations in wheat.

Sterility

In a previous paper (14) it was reported that plants that are heterozygous for any one of three of these same translocations and therefore have an association of four chromosomes at meiosis, show only from 5 to 10% sterility. The additional four translocations included in this study cause approximately the same amount. The data for plants that have various combinations of two translocations and therefore show either two complexes of four or one of six, are given in Table III.

TABLE III

PERCENTAGES OF STERILITY IN PLANTS HETEROZYGOUS FOR TWO DIFFERENT TRANSLOCATIONS

Complexes	Translocations involved	Female		Male	
		Total examined	Sterility, %	Total examined	Sterility, %
1 of 6	$T_1 \times T_3$	275	18.6	5042	24.5
	$T_1 \times T_4$	124	17.0	1025	20.5
	$T_2 \times T_4$	143	29.0	974	36.8
	$T_2 \times T_7$	345	22.8	220	16.4
	$T_3 \times T_4$	80	21.2	431	28.2
	$T_3 \times T_7$	174	25.1	1163	21.4
	$T_4 \times T_7$	209	24.4	1432	23.2
	$T_5 \times T_6$	302	19.5	520	24.4
	$T_5 \times T_7$	148	23.6	421	32.2
2 of 4	$T_1 \times T_5$			612	11.0
	$T_1 \times T_6$	226	13.3	556	13.0
	$T_1 \times T_7$	331	21.4	811	23.0
	$T_2 \times T_3$	175	17.1	1320	22.0
	$T_2 \times T_5$	121	22.8		
	$T_2 \times T_6$	278	11.2	1926	12.7
	$T_3 \times T_5$	220	12.7	801	21.3
	$T_4 \times T_6$	346	11.6	1321	18.5
	$T_5 \times T_7$	287	14.9	315	14.4

It will be observed that in most types with a complex of six, the sterility is between 20 and 30%, but may fall a little below or rise a little above these figures. This is far lower than the 90% that is to be expected if segregation is random and also much lower than the 75% that is to be expected if the only restriction in random segregation is that homologous centromeres must go to opposite poles. A sufficient number of specimens of the different types

were studied both on the male and female sides to prove that at least some of the differences in sterility between the different types were consistent and significant.

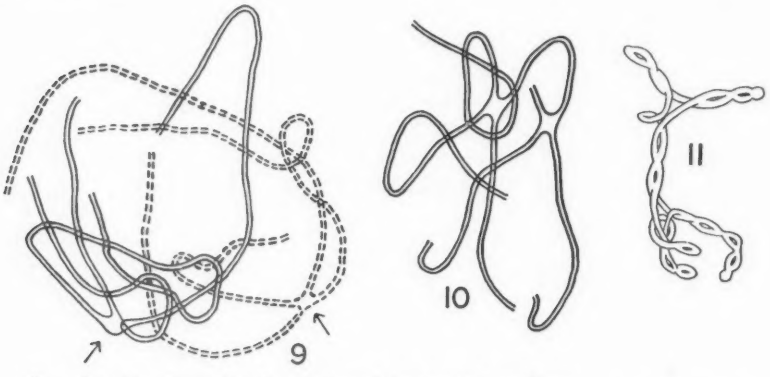
In most plants with two complexes of four chromosomes the sterility is between 10 and 20%. This again is much lower than the 88% to be expected with random segregation or the 75% to be expected if segregation is directed only to the extent that homologous centromeres must go to opposite poles. It is in good agreement with the percentages to be expected on the basis of results obtained with one complex of four.

The reason that plants with one complex of six are somewhat more sterile than those with two complexes of four, will be discussed later.

Chromosome Behaviour

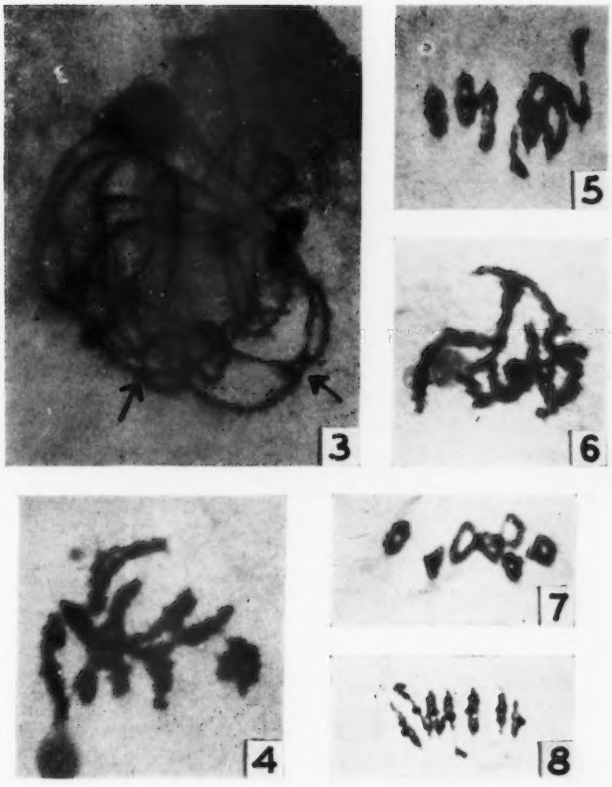
In Complexes of Four

All the chromosomes of *T. monococcum* are so similar in their morphological features, as first described by Kagawa (8), that only with great difficulty and much labour can they be identified separately at mitosis. It is impossible to identify them in meiotic stages. Of particular importance in relation to the fertility of translocation heterozygotes are the facts, (1) that they all have nearly median centromeres, and (2) that the chiasmata are usually terminal at metaphase.



Figs. 9 to 11. *Camera lucida drawings of translocation complexes.*
Fig. 9. *The two complexes of four which were photographed in Fig. 3. In the drawing the bivalents are omitted. The arrows indicate the centres of the crosses.* Fig. 10. *Pachytene stage of an association of six chromosomes in the form of a double cross.* Fig. 11. *Double-cross association of six at diplotene.*

The pachytene stage in a nucleus with two complexes of four and three pairs of chromosomes is photographed in Fig. 3. The centres of the cross-shaped complexes of four chromosomes are indicated by the arrows. The two complexes from this same cell are drawn in Fig. 9, one being represented by solid lines and the other by broken lines; for the sake of clearness the



FIGS. 3 TO 8. *Photographs showing translocation complexes at various stages.* ×*1000.* FIG. 3. *Pachytene stage in a nucleus with two complexes of four chromosomes and three pairs. The centres of the two cross-shaped complexes are indicated by the arrows.* FIG. 4. *Late prophase; two cross-shaped complexes of four (in centre) and three bivalents.* FIG. 5. *Metaphase; figure of eight and zigzag chain at right, three bivalents at left.* FIG. 6. *Prophase; star-shaped complex of six chromosomes plus four pairs. Three of the latter are towards the left and above, one at the right and below, crossing one arm of the star.* FIG. 7. *Metaphase; complex of six in the form of a zigzag ring, and four bivalents.* FIG. 8. *Metaphase; zigzag chain of six, plus four pairs.*

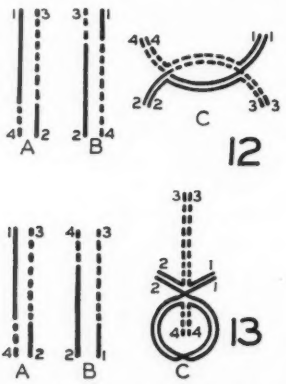
bivalents are omitted in the drawing. It will be observed that the association of homologous parts is close right up to the loci of interchange. This is true of all the different complexes of four observed at pachytene. The cross-shaped configuration is usually readily evident at the central part of the complex, but the long arms are so curved and twisted as to destroy the shape of the cross for the complex as a whole.

An important point in relation to sterility is the position of the breaks that led to the interchange. This can be determined from the relative lengths of the arms of the cross. In Fig. 9 it will be observed that in each complex two arms are about half as long as the other two. Actual measurements of several specimens of one complex give the proportions 1.0 : 1.3 : 2.1 : 2.1. Since the original chromosomes were of nearly equal length and both had median centromeres, the break in one chromosome must have occurred at a point about one-third the distance along one arm from the centromere, and in the other chromosome somewhat nearer the centromere. Examination of other translocations shows a considerable range in the position of the breaks. In the majority all four arms are nearly equal (Fig. 4); in such translocations both segments exchanged must have consisted of nearly complete arms of the original chromosomes and the breaks must have occurred very near the centromere. But in others one arm of the cross is very short and the other three long; in such cases one segment must have been very short and the other long; the break in one chromosome must have been near the end and in the other near the centre. In general it may be said that there is a strong tendency for breaks to occur near the median centromere but various other positions are represented.

The diakinesis stage in a nucleus with two complexes of four and three bivalents is photographed in Fig. 4. With the contraction of the arms the cross configuration becomes more symmetrical. Both complexes are beginning to loosen. It is important to note that the complex usually does not open out into a flat ring but changes more or less directly from the cross configuration to a figure of eight. This is in strong contrast to the behaviour in maize where, according to published accounts, apparently all complexes pass into the flat ring. The difference appears to be associated with a difference in the stage at which the opening out occurs. According to Anderson (1) the separation begins in maize in late pachytene or early diplotene, and at diakinesis only the ends are joined to form a typical ring. In wheat the separation occurs considerably later, and at diakinesis relatively few open rings are visible. Usually the complex appears to pass from a partly open cross directly to a figure of eight.

At metaphase the great majority of complexes are in the form of a horizontal figure of eight, which may readily be mistaken for two bivalents in contact. This is the position for alternate, disjunctional segregation. A small proportion form open flat rings. Some have broken at one point and form zigzag chains. Fig. 5 is a photograph of a cell showing a zigzag chain and a figure of eight at the right and three bivalents at the left.

Both the figure of eight and the zigzag chain must segregate in alternate fashion and therefore produce viable gametes. Of 100 complexes carefully studied for this purpose, 88 clearly had the disjunctional configuration. This agrees well with the data on sterility previously given.



FIGS. 12 AND 13. *Associations of four due to two different translocations involving the same chromosomes.*
FIG. 12. *A, chromosomes resulting from first translocation which involved Ends 2 and 4; B, those resulting from the second translocation which involved Ends 1 and 3; C, the association in a plant heterozygous for both.* FIG. 13. *A, translocation involving Ends 2 and 4; B, involving Ends 1 and 4; C, the association in a plant heterozygous for both.*

A matter that has apparently not been explored is the form that a complex would take in a plant that is heterozygous for two different translocations involving the same chromosomes. Of course if the points at which both chromosomes break are exactly the same in both translocations, no complex but only pairs will be formed in the F_1. But if the points of interchange in either or both chromosomes are different in the two translocations, a single complex of four should appear in a plant that is heterozygous for both. This complex should, however, be very different from the simple cross of a single reciprocal translocation. Fig. 12 illustrates the situation when the two translocations involve the opposite ends of both chromosomes. In the first translocation (Fig. 12A), End 2 of Chromosome 1.2 and End 4 of 3.4 have been exchanged; in the second (B), Ends 1 and 3 have been exchanged. The form that the complex should take in a plant heterozygous for both is shown in Fig. 12C. Fig. 13 illustrates the situation when both translocations (A and B) involve the same end of one chromosome (End 4) but opposite ends of the other (Ends 1 and 2). In the cases illustrated in these figures, both segments exchanged in the first translocation are of the same length, and this length is the same as that of both segments exchanged in the second translocation. But any combination of lengths of these four segments may presumably occur, and any combination of ends. All these numerous variations should theoretically result in complexes of four that would be of different

and often complicated form. Since the chances of both breaks for both translocations occurring at identical points are very small, some such configuration in place of the simple cross or two pairs, is to be expected. In none of the material of *T. monococcum* have the same two chromosomes been involved in two different translocations. But in *T. durum* a case of the type illustrated in Fig. 12 has been produced.

In Complexes of Six

The form that a complex of six chromosomes takes during prophase is usually reported to be a star, as shown in Figs. 2 and 6. Actually this configuration occurs only when the two breaks in the chromosome that is involved in both translocations occur at the same place. If the piece transferred in the first translocation is from the opposite arm to that transferred in the second, the complex of six in the plant that is heterozygous for both will take the form of two crosses with one segment in common, as illustrated in Fig. 14. The double-cross configuration will also be produced if the segments from

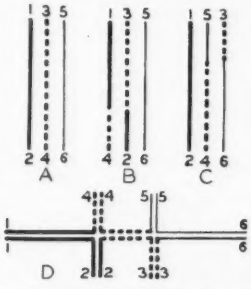


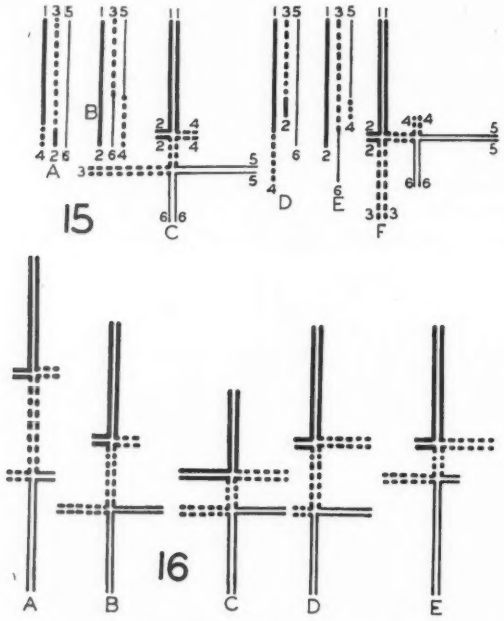
Fig. 14. *A, the original chromosomes; B, after the first translocation; C, after the second; D, double-cross configuration in a plant heterozygous for both.*

the common chromosome that are transferred in the two translocations are from the same arm but are of unequal length. This is demonstrated in Fig. 15. Unless there is a marked tendency for the different breaks to occur at the same place, the double cross should be much more frequent than the star. Of course transitions between the two configurations should occur when the pieces transferred from the same arm are unequal but not very markedly so.

An actual pachytene double-cross configuration is drawn in Fig. 10. All parts are represented in their proper positions and relative lengths, as drawn with the aid of a camera lucida. A diplotene stage is shown in Fig. 11. Naturally the double-cross configuration is usually distorted, particularly in the earlier, uncontracted stages, by the curving and twisting of the arms.

The form of the double cross varies greatly with the lengths of the segments that were translocated, as these determine the lengths of the different arms of the cross. This point is illustrated by the diagrams in Fig. 16, which show a few of the configurations produced from the same three original chromo-

somes by varying the lengths of the translocated segments. In each configuration the chromosome involved in both translocations is the one represented by the broken line, and the two segments translocated from it are from opposite ends. There are similar variations when segments of unequal length are transferred from the same arm of the common chromosome. Two of these are illustrated in Fig. 15.



FIGS. 15 AND 16. *Some forms of double-cross configurations of six chromosomes, depending on the positions and relative lengths of the segments exchanged.*

FIG. 15. *The segments of the common chromosome are from the same end in both translocations. A, first translocation, short segments; B, second translocation, long segments; C, resulting complex; D, first translocation, short–long; E, second translocation, short–long; F, resulting complex.* FIG. 16. *The segments of the common chromosome (broken line) are from opposite ends in the two translocations. Various forms due to differences in the relative lengths of the segments.*

A cell with a star-shaped complex of six and four pairs is photographed in Fig. 6. Usually the star shape of the central portion is readily evident, but the arms are so curved and intertangled as to destroy the star shape for the complex as a whole, particularly at earlier stages. The relative lengths of the arms vary with the length of the segments translocated but the two segments transferred from the common chromosome in the two translocations must be nearly equal or a double cross instead of a star will be produced.

Whatever form the complex of six may take during prophase, it is a zigzag ring at metaphase in the great majority of cases. Fig. 7 shows a cell with

such a ring and the four bivalents. Owing to the failure of a terminal association the ring is sometimes replaced by a zigzag chain as illustrated in Fig. 8.

Because of the zigzag form, whether of ring or chain, which is typically present at metaphase, it is obvious that segregation of complexes of six is normally alternate. This is true of the complexes in all the nine F_1 combinations that have been studied. Spores resulting from such segregation should be competent. Of 75 complexes carefully examined at metaphase 56 (74.6%) showed the complete disjunctional arrangement.

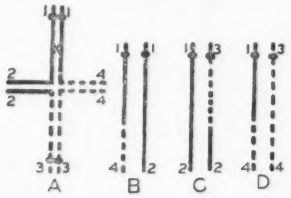


FIG. 17. *Effect of crossover between the centromere and the point of interchange. A, prophase configuration with a crossover in Arm 1; B, chromosomes resulting from the crossover; C and D, chromosomes of the spores that would result from alternate segregation. Both have a deficiency and a duplication.*

A considerable number of offspring (F_2) of plants that had complexes of six (F_1) were examined. In every pollen mother cell examined at a suitable stage in all these plants there were either seven pairs of chromosomes or a complex of six and four pairs. Therefore, if any gametes are formed through non-disjunctional segregation with more or fewer than the normal number of seven, or with the normal number but abnormal constitution, they are unable to produce viable offspring. Only two types of F_1 gametes function successfully; one has one of the two translocational arrangements that went into the cross and the other has the second. F_2 plants with seven pairs were fully fertile; those with complexes of six showed an average sterility of 21.1%, in close agreement with F_1.

Discussion

The cytological studies show that the reason for the lack of sterility in wheat plants which are heterozygous for a translocation is that segregation is not random but directed. It is directed not merely by the movement of homologous centromeres to opposite poles, but also as a rule by the movement in opposite directions of adjacent chromosomes that have non-homologous centromeres. If such alternate segregation were invariable, only fully competent spores would be produced and fertility would be complete. But, in a small percentage of cells with associations of four and, in a somewhat larger percentage with associations of six something interferes with this normal procedure or in some other way causes sterility.

In order to explain why segregation is regularly disjunctional, it is only necessary to assume that the repulsion that causes segregation involves the

whole bodies of chromosomes and not merely the centromeres as is often postulated. If every chromosome repels those on either side of it, the alternate movement would result. And if the repulsion of the two homologous members of ordinary pairs involves more than the centromeres, it is to be expected that every chromosome in the ring would repel its neighbour on either side because adjacent portions of adjacent chromosomes are homologous, as shown by the fact that they are closely paired in prophase to form one arm of the cross.

On the other hand, in order to explain regular alternate segregation on the basis of centromere repulsion alone, it is necessary to assume that every centromere is influenced by one that is not homologous with it. Any centromere in the ring is homologous with that of the chromosome on one side only and yet it must be influenced by the one on the opposite side also, if alternate segregation depends on the centromeres. But there is no reason to think that any centromere can influence the direction of movement of any non-homologous centromere. Mendel's principle of independent assortment implies that there is no such influence. The normal occurrence of alternate segregation from translocation complexes therefore shows that the forces that cause chromosome segregation involve whole chromosomes (or at least their ends) and not merely the centromeres. Presumably this is true of chromosome pairs as well as complexes.

If the alternate segregation in wheat, followed by a high degree of fertility, is understandable on these general conceptions, there is immediately encountered the difficulty that in *Zea* there is 50% sterility and, according to McClintock (9), adjacent as well as alternate segregation. The situation in *Pisum* is similar, according to Håkansson (7). It is not to be expected that the basic mechanism of chromosome movement would be so different in different genera that one would show only alternate and another random segregation. It is more reasonable that the segregation is basically alternate (disjunctional) but that in some cases special conditions interfere and give the appearance of random segregation.

This opinion is supported by several considerations:

(1) In maize the segregation is not really random, for, if it were, the sterility would be 66.6 instead of 50% in cases of rings of four and 90 instead of 70% in cases of rings of six. Of course it may be maintained that randomness is restricted only to the extent that homologous centromeres must go to opposite poles. But once the complexes have opened out into rings, there is no apparent reason why homologous centromeres must necessarily go to opposite poles. Moreover, McClintock (9) showed that they may actually go to the same pole.

(2) The translocations that have been studied in maize were discovered *because* they cause semisterility. Search is made for them by examining semisterile plants. Consequently, only those with non-alternate segregation are discovered. It is possible that there may be others with alternate segregation, as in wheat, that have not been found. On the other hand the trans-

locations in wheat were discovered through a systematic cytological examination of the offspring of radiated plants (the only way they could be discovered) and their fertility was proved later. It would be of interest to know whether a systematic cytological examination of the offspring of radiated maize would reveal the occurrence of fully fertile translocation heterozygotes in that genus. In this connection it is important that Clarke and Anderson (4) mention the occurrence in maize of a few translocations involving sterility from 19 to 50%, and Burnham (3) reports a case with 25%.

(3) In certain other genera disjunctional segregation is the rule or is interfered with only enough to produce less than 50% sterility. The zigzag segregation of the large rings of *Oenothera* is well known. Gairdner and Darlington (6) found 30% non-disjunction in a case in *Campanula*. Sax and Anderson (11) reported that different translocations in *Tradescantia* exhibit different amounts of adjacent segregation. According to Bergner, Satina, and Blakeslee (2) most of the translocation heterozygotes in *Datura* are fully fertile. In different translocations in the same and in different genera, alternate segregation seems to be prevented to different degrees. Even in *Triticum* disjunctional segregation is not invariable and fertility is not quite complete.

Factors that cause sterility by interfering with alternate segregation or in some other way, should be revealed by comparing, with respect to chromosome structure and behaviour, those forms like maize and peas that show semisterility with those like wheat that do not. Such a comparison brings out the following points:

(1) In the bivalents of maize there are numerous interstitial chiasmata, and the same is true in *Pisum*. In wheat, on the other hand, both in bivalents and complexes, the chiasmata are usually terminal, although a few interstitial ones may be found. The situation is the same in *Oenothera*, *Datura*, and *Campanula*, the other genera in which examples of alternate segregation or low sterility have been reported. As Darlington (5) pointed out, the occurrence of interstitial chiasmata would interfere with the opening out and orientation of the complex on the metaphase plate. On the other hand the data of Sax and Anderson (11) from *Tradescantia* fail to reveal a correlation between the number of interstitial chiasmata and the amount of non-disjunctional segregation. Their cases may, however, have been complicated by another factor to be considered later.

(2) In spite of the larger number of interstitial chiasmata in maize, the cross appears to open out earlier than in wheat. According to Anderson (1) the opening out begins in maize in late pachytene or early diplotene and by diakinesis only the ends of the chromosomes are in contact, so that open flat rings are characteristic of diakinesis. Perhaps the examples on which these observations were made were ones without the interstitial chiasmata which would be expected to prevent the opening out. In wheat, although there are fewer interstitial chiasmata, relatively few flat rings are to be seen at diakinesis. Usually the complex seems to change directly from a cross to a

figure of eight without passing through an open-ring stage. If the repulsive forces operate strongly at an early stage and produce a flat open ring before orientation has begun, as appears from published descriptions to be the rule in maize, there might be little tendency for adjacent chromosomes to move in opposite directions. But if the repulsion of homologous parts takes place only later, after orientation has begun, the figure of eight would naturally be formed as in wheat, and adjacent chromosomes must go in opposite directions. It is clear, however, that this cannot be a general explanation for all types, because in *Oenothera* the complexes regularly pass into very large, widely open rings and thence into the zigzag condition.

(3) In most of the chromosomes of maize the centromeres are not median; the same is true in *Pisum*. In *Triticum monococcum*, on the other hand, all seven have median centromeres as have those of *Datura* (10), and most of those of *Campanula* and *Oenothera*. When the centromere is in the middle of the chromosome, the distance between it and the point of interchange is likely to be less than when it is near the end. Consequently, crossovers are likely to be fewer between a median centromere and the point of interchange. Fig. 17 shows that chiasmata in this region must have difficulty in terminalizing, and, when they do, all the gametes resulting from alternate segregation will be inviable. The position of the centromere near the end of the chromosome therefore tends to cause sterility through the occurrence of crossing over between the centromere and the locus of interchange.

The same effect would be produced even though the centromere were median, if at least one of the segments translocated were short. A short segment necessarily means a considerable distance between centromere and point of interchange, and hence more frequent crossovers in that region. But there is no reason to expect that the length of the segments exchanged should be greater in wheat than in maize or peas. As a matter of fact in most of the translocations studied in wheat the segment consisted of nearly a whole arm. But in some the segment was very short, and yet the sterility was low.

If the chromosome as a whole is short, the distance between centromere and locus of interchange is likely to be short, and hence the frequency of crossovers in that region low. This may be a factor in *Oenothera* and *Datura* which have small chromosomes, in contrast with *Zea*, *Pisum*, and *Tradescantia* which have long ones. But *Triticum* also has long chromosomes.

Thus some of the factors that may be expected to cause sterility in translocation heterozygotes are (1) slow or incomplete terminalization of chiasmata, (2) early separation of homologous parts, (3) non-median position of the centromere, (4) shortness of segments exchanged, (5) great length of chromosomes. Since these factors are variable from species to species, and some of them from case to case within a species, it is to be expected that the degree of sterility will vary. The 50% frequently reported has no special significance. Wheat has only isobrachial chromosomes; its chiasmata are mostly terminal; in most of the translocations studied the segments exchanged are long; and

the separation of homologous parts is delayed so that usually the open ring is omitted. Hence sterility in wheat is low. On the other hand maize has several heterobrachial chromosomes in which many interstitial chiasmata occur and open rings are regularly formed; hence numerous cases with high sterility are observed.

In complexes of six or more chromosomes additional factors come into play. This is evident from the fact that plants with one complex of six are consistently more sterile in wheat than those with two complexes of four, although in both types only two interchanges are involved. Presumably the greater unwieldiness of the larger complexes makes regular orientation more difficult. In addition, it should be remembered that the configuration will usually be a double cross and not a star, unless there is a marked tendency for different breaks to occur at the same locus. In the double cross each of two chromosomes must pair with three others, and this may involve difficulties. Moreover, crossovers in the segment that connects the two crosses will interfere with orientation and will always produce incompetent combinations of chromosomes.

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HEPATOGENESIS OF THE ATLANTIC SALMON (*SALMO SALAR* L.)[1]

BY HELEN I. BATTLE[2]

Abstract

The anlage of the liver of the Atlantic salmon, *Salmo salar* L., arises during the eleventh week, 4.0 mm. embryo, as a proliferation of entodermal cells from the ventral wall of the anterior intestinal region. This anlage soon takes on a double appearance caudally. In embryos of 12 to 12½ weeks (4.7 to 5.0 mm.) it consists of right and left primordia with a common ventral opening into the intestine. By 13 weeks (5.4 mm. embryo) the anlage has curved toward the left, a position assumed through the counter-clockwise rotation of the intestine. The cells are becoming arranged in cords. A subsequent clockwise rotation of the intestine, apparent first in embryos of 14 weeks (6.5 mm.), draws the anlage to a dextro-ventral position. By 16 weeks (8.5 mm.) the ductus choledochus enters the right side of the intestine; the anlage itself passes posteriorly along the ventral surface of the intestine then abruptly forward in the form of a hook to the right of the intestine. The liver cells are arranged as branching trabeculae with some indication of vascularization. The liver anlage becomes rhomboidal and migrates to a position on the right of the rectilinear intestine, where it remains until some time after hatching. In the 25 mm. alevin (26 days after hatching) the intestine through a process of flexion commences to differentiate into cardiac and pyloric stomach, ascending and descending limbs of intestine. Simultaneously the papilla duodeni marking the opening of the ductus choledochus into the intestine is carried to a sinistrodorsal position opening into the ascending limb of the intestine at the level of the second row of pyloric caeca. Subsequent clockwise rotation of the ascending limb finally carries it to the sinistroventral margin. Yolk absorption now permits the liver to grow ventrad to the intestine, while the anterior progression of the intestinal loop is instrumental in its migration to the anterior end of the coelom. In the early parr the liver is somewhat triangular in shape with the apex posterior and in the mid-line. Through unequal growth the left median lobe in the late parr becomes much longer than the right, from which, however, it is never distinctly divided. It has now assumed the general appearance of the organ in the smolt and adult. Enlargement of the blind end of the ductus choledochus in the 21 week embryo (11.8 mm.) forms the gall bladder. Hepatic ducts are formed as branches of the primary ductus choledochus.

Introduction

The development of the liver of the teleostean fishes has been less extensively investigated than has that of other vertebrate types that serve more commonly as laboratory forms for embryological studies. This is particularly true for the Atlantic salmon (*Salmo salar* L.) since the literature reveals a single brief reference by Ziegler (22) to the initial appearance of this organ in the embryo. A survey of the development of the liver of this anadromous species thus offers many problems of interest, not merely during the embryonic phases, but also following the larval period when it undergoes an enlargement and a differential growth which accompanies the metamorphosis of other

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structures already observed as constituting the transition from parr to smolt. The present paper concerns gross morphological changes in the liver during development from the initial appearance of the anlage in the 4 mm. embryo to the adult. A later paper will deal with the simultaneous histogenesis.

Materials and Methods

The embryological materials used in the following study were obtained from a series of salmon embryos collected at the Birch Cove Hatchery of the Atlantic Biological Station, St. Andrews, N.B., during the winter of 1939–1940. The collection consisted of daily samples of stages from fertilization, on November 17, to embryos almost ready to hatch, on May 20. Fixation alternated daily between Bouin's fluid and Davidson's fluid, a formol–glycerine–alcohol–acetic mixture. The latter fixative prevented excessive hardening of the yolk material, and was consequently more satisfactory than Bouin's for material that required to be sectioned. The average temperature over the incubation period was 1.4° C., but, with the exception of four days, a minimum temperature of 0.5° to 1° C. was maintained from November 22 to April 23. For this reason the early developmental stages in this series are passed through at a considerably slower rate than are those in the adjacent St. John hatchery where the minimum temperature is of shorter duration.

Since development is a function of both temperature and time, Wallich (20) designated the age of fish embryos by what he termed "thermal units". One thermal unit (*t.u.*) corresponds to a temperature of 1° F. above 32° F. for a period of 24 hr., or is really a Fahrenheit-calorie–day. For example, a mean temperature of 37° F. for one day yields five thermal units. It is possible, when the incubation period for any series is known in days, as well as

TABLE I

AGE–LENGTH RELATIONSHIP IN DEVELOPING SALMON FROM BIRCH COVE HATCHERY, 1939–1940

Age, wk.	Length, mm.	Water temperature, °C.	Thermal units, Fahrenheit-calorie–day
(Nov. 17, 1939) 0	0.0	1.0 – 3.0	
1–10	0.0–3.6	0.5 – 3.0	
11	4.0	0.5 – 0.6	117.6
12	4.3	0.5 – 0.6	124.6
12 3/7	4.7	0.5 – 0.6	127.7
12 4/7	5.0	0.5 – 0.6	128.7
13	5.4	0.5 – 0.6	131.7
14	6.5	0.6 – 0.7	140.0
15	7.0	0.6 – 0.7	148.0
16	8.5	0.5 – 0.7	158.1
17	9.4	0.6 – 0.7	166.0
18	9.9	0.5 – 0.7	173.6
19	10.6	0.5 – 0.7	182.2
20	11.4	0.6 – 0.7	190.4
21	11.8	0.6 – 1.1	201.1
22	12.4	0.6 – 2.8	217.7
23	14.7	0.7 – 4.5	238.3
24	15.0	1.8 – 5.1	287.3
25	16.4	3.5 – 7.0	356.5
26	19.0	4.6 – 9.5	439.2

the mean daily temperature, to calculate the thermal units, thus expressing the age of an embryo as the sum of both time and temperature.

The age in weeks and in thermal units, the length (i.e., the average for eight individuals) and the temperature of the water are given in Table I for representative embryos studied.

Salmon alevin and fry were collected at two-day intervals at the St. John hatchery, New Brunswick, during May and June, 1939, and fixed in Bouin's fluid. The hatching took place approximately 26 weeks after fertilization. Their age and length relationships together with the average daily water temperature are given in Table II. The length measurements represent the average for 10 individuals.

TABLE II

AGE–LENGTH RELATIONSHIP IN SALMON ALEVIN AND FRY FROM ST. JOHN HATCHERY, 1939

Age (from hatching), days	Length, mm.	Water temperature, °C.	Age (from hatching), days	Length, mm.	Water temperature, °C.
(May 3, 1939) 0	18.5	4.7	(May 3, 1939) 24	25.0	15.0
2	20.0	4.7	26	25.0	14.4
4	20.5	5.3	28	25.0	14.2
6	21.0	5.3	30	25.5	14.2
8	21.5	8.1	32	25.5	13.9
10	22.0	9.5	34	26.0	14.7
12	22.5	10.3	36	26.0	15.3
14	23.0	10.3	38	26.0	16.2
16	23.5	10.9	40	26.5	17.8
18	24.0	12.6	42	26.5	17.5
20	24.5	12.8	44	27.0	15.3
22	24.5	14.5			

Formalin preserved specimens of late fry, early and late parr, smolt, and adults from the Margaree and Moser Rivers, Nova Scotia, and the St. John River, New Brunswick, as well as pregrilse taken from weirs on the Bay of Fundy were also examined.

Serial transverse sections of a carefully graded series of embryos, alevin, and fry were cut at 10 μ and stained with Ehrlich's haematoxylin and triosin. When the salient developmental stages were determined, one to two additional series were prepared in each instance for corroborative study. Because of the relatively large size of the embryos it is possible to observe the liver anlage even at very early stages by microdissection, using fine glass needles. Four to six specimens of a graded series corresponding to those used for the serial sections were dissected for each stage. The graphic method of reconstruction was used to determine the development of the biliary duct systems as well as the extent of the earliest anlagen.

As is well known the size of the embryo and alevin affords no absolute criterion of the state of the development of the fish. Thus in order to permit a careful comparison of the different specimens with one another and with the descriptions of other observers, in the following account the salient details of the formative stage of several structures besides that of the liver are given in each instance to present a general developmental picture.

Development

Initial Appearance of Anlage

The literature dealing with the organogenesis of the teleostean liver is concerned chiefly with its origin and very early differentiation. A number of investigators have observed the first anlage as a single solid ventral outgrowth of the intestine. Vogt (19, p. 155) briefly referred to the initial appearance of the liver in *Coregonus palaea* (Cuv.) as an accumulation of cells beneath the intestine at the level of the pectoral fins. Reighard (11) examined the wall-eyed pike, *Stizostedion vitreum* (Mitchill) and in one instance states that the liver is first apparent as a solid outgrowth from the ventral wall of the alimentary canal just posterior to the pharynx (p. 34); and in another, he states that the liver is formed as one or more hollow branching outgrowths from the tube (p. 61). Lereboullet (6) was the first to observe the liver in the trout (species?) embryo as a little cellular mass against the external wall of the stomach. Balfour (1, p. 78), Stöhr (17), and Laguesse (5) also using the trout (species?) noted that the earliest stage is a solid ventral diverticulum of entodermal cells from the duodenal region of the intestine. Göppert (4) considered that it had more the character of a sac-like protrusion in the salmon trout (*Trutta trutta* L.). Stricker (18) examining the latter species and Riggert (12), *Trutta fario* L. and *Trutta iridea* (Gibb.) corroborated the earlier observations of the solid character of the anlage. Price (10) recorded its appearance as a ventral outgrowth from the mid-gut in the whitefish, *Coregonus clupeaformis* (Mitchill).

In contrast to these observations Wilson (21) indicated the anlage as a solid outgrowth of the dorsal wall of the enteron just posterior to the pectoral fins in *Serranus atrarius* Jord. and Gibb. As it increases in size he observed its ventral growth between the ectoderm and the yolk sac. The possibility of a misinterpretation occurs here, since in a 100-hr. embryo this anlage is a dorsolateral thickening of the intestine, while by 112 hr., the liver lies directly on the opposite side of the intestine extending ventrally. Again, however, at 160 hr. a definite dorsal attachment to the intestine appears although the organ extends laterally to the ventral body wall.

A liver anlage arising from the lateral surface of the intestine has been observed in a number of teleosts. Chevey (3) found a ventrolateral location of the anlage in a nine day old embryo of the perch (*Perca fluviatilis* L.). He assumed, however, that it was originally ventral since the intestine had undergone some torsion. Ballantyne (2), in a general embryological survey of *Callichthys littoralis* Hancock, observed the first rudiment of the liver as a little pouch from the lateral wall of the alimentary canal just where it bulges to the left into the stomach rudiment. Pohlmann (9) states that the liver appears as a lateral thickening of the intestine in *Trichopodus trichopterus* (Pall). Through torsion of the intestine it comes to lie ventrally, pressing into the yolk. She found a similar condition in *Cichlasoma bimaculatum* L. and *Cichlasoma cutteri* Fowler, where the left lateral anlage is not a thickening

but an actual protrusion of the intestine. Through torsion of the latter the anlage becomes ventrolateral, the ductus choledochus migrating ventrally toward the right.

Embryo: 4.0 mm., 11 Weeks, 117.6 Thermal Units

No evidence of a liver anlage could be found in embryos from Birch Cove hatchery 10 weeks after fertilization. In the 4.0 mm. 11 week old embryo the anlage is readily identified as a proliferation of cells from the midventral wall of the gut (Figs. 1, 16).

The blastoderm in a 4 mm. embryo has overgrown slightly more than three-quarters of the yolk sphere, giving the appearance of a large yolk plug. Twenty-eight to 30 pairs of mesodermal somites are present. The brain region has expanded considerably over the posterior portion of the neural cord. Three primary divisions are evident, the forebrain, midbrain, and hindbrain. The optic anlage now a shallow cup lies lateral to the forebrain with which it is connected by the optic stalk. The lens placode is present opposite the mouth of the cup. The auditory placode has sunk deeply below the surface and has become almost completely cut off as a hollow sac, the auditory vesicle. No lumen is present in the pharyngeal or intestinal regions of the gut with the exception of short irregular distances which are patent in the intestine. The notochord is solid, its cells not yet appearing vacuolated. The appearance of the anlage at this stage is considerably in advance of that noted by several investigators for the trout. Laguesse (5) observed its appearance after closure of the blastopore and Stricker (18) noted it first in *Trutta trutta* L. when the posterior body segments were already extending freely beyond the yolk sphere and the head lifted free of the yolk.

The anlage commences anteriorly as a midventral rather blunt cellular projection of the pharynx at the mid-level of the auditory vesicle, a position that Stöhr (17) had previously noted for the trout. At this region it has an approximate depth of 0.06 mm. Passing posteriorly it extends for a distance of about 350 μ and becomes progressively deeper as the intestinal region is attained. In a typical transverse section of this region (Fig. 16) the intestinal wall is composed of columnar epithelial cells with oval or elliptical nuclei. Those composing the cellular mass of the anlage have somewhat more rounded nuclei, and are extremely active mitotically. The lumen of the intestine is a small spherical pore in some regions, a narrow vertical slit in others, and elsewhere not yet patent. The intestine and anlage at this level are 0.16 mm. in depth, 0.11 mm. of which may be considered to represent the primordial liver. A heavy layer of splanchnic mesoderm is apparent on either side of the anlage. Its cells become more flattened as they pass over the surface of the yolk. The ventral cells of the anlage rest directly on the yolk syncytium, in which very large chromatin laden nuclei are present.

Double Character of Anlage

The majority of investigators have observed a single liver anlage. Siwe (15) and Smallwood and Derrickson (16) believe that they can detect a double anlage. The latter condition regularly occurs in *Acanthias*, an elasmobranch, in which the liver arises as a pair of shallow lateral diverticula from the lateral walls of the ventral half of the gut (13). Siwe (15) described the development of the liver in *Trutta fario* L., and in *Cyprinus* and *Syngnathus* embryos. In the trout the anlage makes its first appearance 15 3/24 days after fertilization as a thickening of the intestinal wall directly cranial to the yolk sac. By 16 9/24 days it has developed into two primordia (ventral saccate evaginations) with a common midexcretory duct. For a relatively long time the two primordia are cranially separated and fused caudally in the median duct. Smallwood and Derrickson (16) found that the liver in *Cyprinus carpio* L. originates as two ventrolateral evaginations from the mid-gut at the time of hatching. Maxia (8), however, observed the origin of the liver in this species at a considerably earlier period prior to hatching, when it is first apparent as a solid mass of endodermal epithelium along the lateral intestinal wall.

Embryo: 4.7 mm., 12 3/7 Weeks, 127.7 Thermal Units

Embryos at this stage possess approximately 38 to 40 somites. The blastopore has closed, its position being indicated only by a slight furrow on the yolk sac in the caudal region. The embryo is prominently elevated above the yolk sphere and occupies about one-third of its circumference. The lens

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FIGS. 1 TO 3 *are from graphic reconstructions;* FIGS. 4 TO 13 *from camera lucida drawings of dissections.*

FIG. 1. *Relationship of primary liver anlage to intestinal epithelium in the 4.0 mm. embryo. Ventral and right lateral aspects. Photomicrograph through the level X will be found in Plate I, Fig. 16.*

FIG. 2. *Relationship of primary liver anlage to intestinal epithelium in the 4.7 mm. embryo. Right lateral aspect. Photomicrograph through the levels X will be found in Plate I, Fig. 17.*

FIG. 3. *Relationship of the liver anlage and the intestinal epithelium in the 5.4 mm. embryo. Ventral and right lateral aspects. Photomicrographs through the levels X_1, X_2, and X_3, will be found in Plate I, Figs. 19, 20, 21.*

FIG. 4. *Ventral aspect, intestinal region of 6.5 mm. embryo showing first expansion of liver anlage toward the right.*

FIG. 5. *Ventral aspect, intestinal region of 8.5 mm. embryo showing migration of ductus choledochus, and whole liver anlage toward the right. Photomicrographs through the levels X_1 and X_2 will be found in Plate II, Figs. 22, 23.*

FIG. 6. *Ventral aspect, showing intestine and rhomboidal-shaped liver of 11.8 mm. embryo. Photomicrograph through the level X will be found in Plate II, Fig. 24.*

FIG. 7. *Ventral aspect of intestine and liver of 14.7 mm. embryo.*

FIG. 8. *Ventral and dorsal aspects of the intestine and liver of 22.5 mm. alevin (larva).*

FIG. 9. *Ventral aspect of intestine and liver of 25 mm. alevin (larva).*

FIG. 10. *Ventral aspect of digestive tract of 26 mm. alevin (larva).*

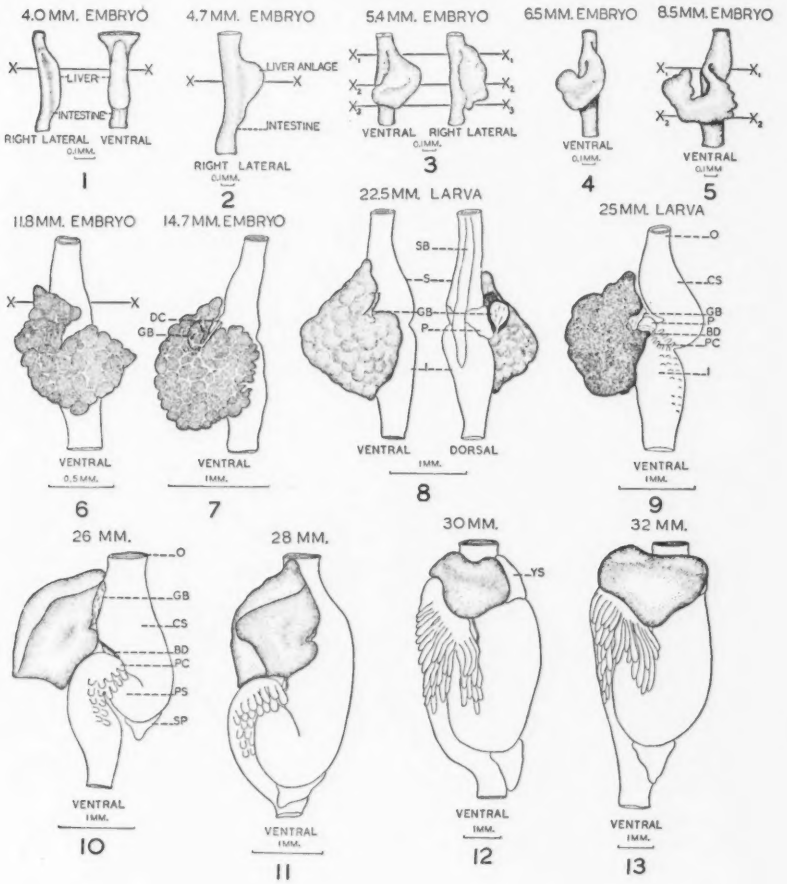
FIG. 11. *Ventral aspect of digestive tract of 28 mm. fry.*

FIG. 12. *Ventral aspect of digestive tract of 30 mm. fry.*

FIG. 13. *Ventral aspect of digestive tract of 32 mm. early parr.*

Abbreviations: C S = *cardiac stomach;* D C, B D = *ductus choledochus, common bile duct;* G B = *gall bladder;* I = *intestine;* O = *oesophagus;* P = *pancreas;* P C = *pyloric caeca;* P S = *pyloric stomach;* S = *stomach;* S P = *spleen;* S B = *swim bladder.*

has separated from the surface ectoderm and has migrated into the mouth of the optic cup. The auditory vesicle has enlarged and has sunk below the surface. The brain has increased in size. The cerebrum, pineal body, and infundibulum have arisen from the forebrain. The midbrain has formed the two optic lobes, while the hindbrain has differentiated into the metencephalon and myelencephalon. The dorsal fin fold extends from the head region posteriorly, surrounding the caudal region (knob) as the fore-runner of the caudal fin. Pectoral fins have appeared as lateral extensions of the body wall at the level of the first somites. The notochord is prominent with a few cells commencing to show the initiation of vacuolation as indicated by the peripheral migration of nuclei. Three pairs of dorsolateral branchial folds are formed on either side of the pharynx somewhat anterior and ventral to the ear. The



pharynx is still occluded, but posterior to it the gut now possesses a narrow dorsoventrally elongated lumen, which, however, is only intermittent along its course.

The anlage of the liver at this stage (Fig. 2) is about 0.4 mm. in length. As it projects ventrally it appears low cranially but rapidly becomes deeper. Its caudal margin declines gradually as it passes posteriorly and dorsally. In this respect it differs from *Salmo fario* L. (18), in which the caudal margin is directed very steeply dorsad. It is separated from the intestine by a very shallow longitudinal furrow. The anlage commences about 30 μ behind the auditory vesicle where it appears, as a somewhat blunt median ventral cellular proliferation about 0.08 mm. in depth. Passing posteriorly for about 60 μ it becomes a heavier cellular proliferation located ventral to the intestinal lumen and with a depth of 0.12 mm. It is composed of two or three layers of low columnar cells, sometimes irregularly arranged, in other instances radially. The nuclei are oval and active mitotically. Approximately 90 μ behind this (Fig. 17) the anlage takes on a double character with the left diverticulum appearing slightly anteriorly to the right. The maximal diagonal depth of either diverticulum from the intestinal lumen is about 0.11 mm. The cells at the base of each tend toward a radial arrangement but offer no distinct cytological differences from those forming the intestinal wall itself. They rest directly on the yolk syncytium. The splanchnic mesoderm covering the anlage laterally is of a low columnar type arranged in two or three layers. As it passes over the yolk surface the epithelium gradually becomes flattened and reduced to a single layer in thickness.

Embryo: 5.0 mm., 12 4/7 Weeks, 128.7 Thermal Units.

The anlage in this embryo is considerably advanced over the 4.7 mm. specimen. The pronephric duct has appeared and a vertically elongated intestinal lumen is present anteriorly along the course of the single anlage. Posteriorly the lumen appears triangular because of the entrance into it ventrolaterally of the ducts from the double anlage. For a distance of 0.15 mm. from its anterior extremity, the anlage has the character of a single cellular proliferation but posterior to this for about 0.26 mm. it takes on a double character (Fig. 18). The right diverticulum is somewhat smaller than the left having a maximal diagonal depth of 0.18 mm. from the base of the intestinal lumen, as compared to 0.23 mm. for the left. Consequent upon this, the whole structure is beginning to shift slightly toward the left. The epithelial cells composing the intestinal wall now appear to be pseudo-stratified columnar. Those of the solid anlage are characteristically low columnar and are rather irregularly arranged. The nuclei have become somewhat vesicular but are still actively mitotic.

These conditions occurring in the salmon differ from those observed by Siwe (15) for *Trutta fario* L., where the two diverticula were observed to be separate cranially and fused caudally. In the salmon they appear single or fused cranially and are distinct from one another caudally.

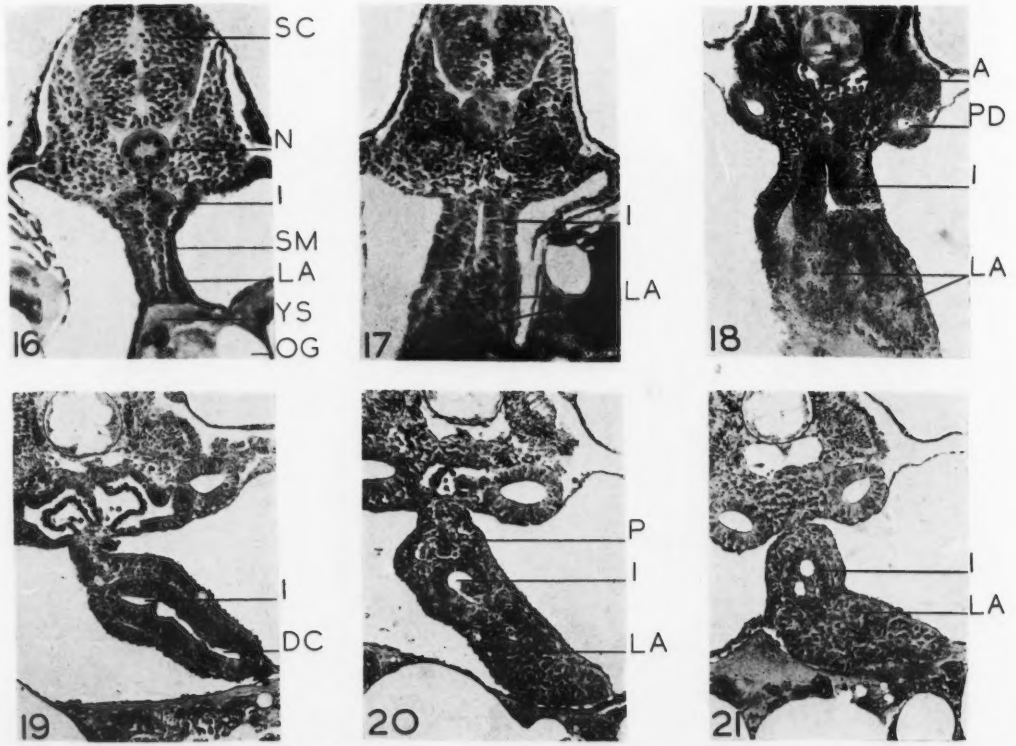


PLATE I

Photomicrographs of transverse sections of salmon embryos illustrating the origin and differentiation of the liver. FIG. 16. *An 11 week old (4 mm.) embryo showing primary liver anlage at level X of Fig. 1.* ×125. FIG. 17. *A 12 3/7 week old (4.7 mm.) embryo at level X of Fig. 2.* × 125. FIG. 18. *A 12 4/7 week old (5.0 mm.) embryo through mid-level of liver anlage.* ×115. FIG. 19. *A 13 week old (5.4 mm.) embryo at level X_1 of Fig. 3.* ×115. FIG. 20. *A 13 week old (5.4 mm.) embryo at level X_2 of Fig. 3.* ×115. FIG. 21. *A 13 week old (5.4 mm.) embryo at level X_3 of Fig. 3.* ×115.

Abbreviations: A = *aorta;* D C = *ductus choledochus;* I = *intestine;* L A = *liver anlage;* N = *notochord;* O G = *oil globule in yolk;* P = *dorsal pancreas;* P D = *pronephric duct;* S C = *spinal cord;* S M = *splanchnic mesoderm;* Y S = *yolk syncytium.*

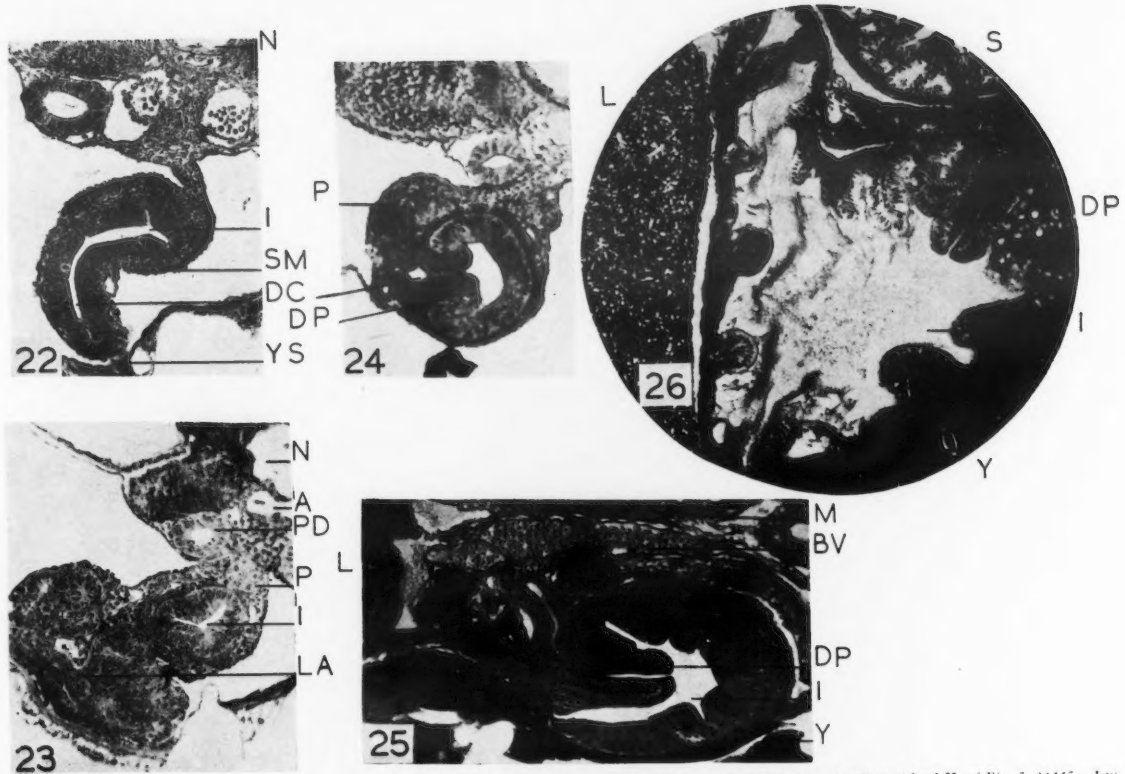


PLATE II

Photomicrographs of transverse sections through salmon embryos at levels of developing liver. FIG. 22. *A 16 week old (8.5 mm.) embryo at level X_1 of Fig. 5.* ×115. FIG. 23. *A 16 week old (8.5 mm.) embryo at level X_2 of Fig. 5.* ×115. FIG. 24. *A 21 week old (11.8 mm.) embryo at level of papilla duodeni (X of Fig. 6).* ×90. FIG. 25. *Intestine of 22.5 mm. alevin (12 days after hatching) at level of papilla duodeni.* ×115. FIG. 26. *Intestine, stomach, and liver of 26 mm. alevin (36 days after hatching) at level of papilla duodeni.* ×115.

Abbreviations: A = *aorta;* B V = *blood vessel;* D C = *ductus choledochus;* D P = *papilla duodeni;* G B = *gall bladder;* I = *intestine;* L A = *liver:* N = *notochord;* M = *musculature:* P = *dorsal pancreas:* P D = *pronephric duct:* S = *stomach;* S M = *splanchnic mesoderm;* Y = *yolk;* Y S = *yolk syncytium.*

Torsion of the Anlage Toward the Left

Embryo: 5.4 mm., 13 Weeks, 131.7 Thermal Units

In an embryo at this stage of development approximately 50 somites are present. The head and tail regions have become raised off the yolk surface. The brain has increased greatly in volume, more especially by growth of the optic lobes and cerebellum. The optic cup now appears as a flattened sphere with a large cavity filled by the lens. Pectoral fins are well developed, and the median fin fold is continuous around the now laterally flattened tail, from the dorsal to the ventral median line. Vacuolation of the cells in the central region of the notochord is taking place. The lumen of the intestine appears about 0.3 mm. behind the ear. It is, however, quite irregular sometimes disappearing, at others becoming double.

The liver anlage (Fig. 3) once more appears as a single structure through union of the diverticula described in the preceding stages. It is still indistinguishably fused throughout its length of 0.43 mm. with the ventral wall of the intestine, but is no longer directed precisely ventrad, being folded over toward the left except at the posterior end. The original right lateral surface has become ventrolateral, the original left has become dorsolateral. This has been brought about by a counter-clockwise rotation of the intestine about its longitudinal axis, when viewed from the anterior end. Reference to transverse sections of the anlage at various levels (Figs. 19, 20, 21) will corroborate this. The anterior tip of the anlage appears 0.63 mm. posterior to the auditory vesicle approximately at the level of the anterior termination of the pronephric duct. The intestinal lumen (Fig. 19) is now no longer vertical, but through rotation of the intestinal tube has become horizontal. The ductus choledochus with a partially occluded lumen enters it on the left lateral border. The lining epithelial cells of both intestine and ductus are pseudostratified low columnar and are separated from the thickened splanchnic mesoderm by a very thin layer of connective tissue. The splanchnic mesoderm becomes flattened progressively from dorsal to ventral and that from either side meets to fuse ventral to the ductus choledochus, thus separating the latter from the yolk syncytium. About 0.1 mm. posterior to the previously described section, the intestinal lumen becomes irregular (Fig. 20). The splanchnic mesoderm is still flattened ventrally and serves temporarily as a thin separating layer between the hepatic cells and the yolk syncytium. The lumen disappears intermittently and may reappear in some irregular form such as the double character shown in Fig. 21, a section about 0.26 mm. from the anterior end of the anlage. Rotation of the intestine has not taken place to any extent at this level as indicated by the fact that the anlage still remains ventral, directly overlying the yolk syncytium. Had rotation taken place the splanchnic mesoderm could have grown medially from either side to meet ventral to the anlage. The cubical to low columnar hepatic cells are loosely arranged in the form of cords. There is some indication of loose connective tissue between them but no vascularization. The anlage at this level has a maximum diameter of 0.22 mm.

Although several investigators studied hepatogenesis in the trout, only Stricker (18) using *Trutta trutta* L. observed a temporary torsion of the early anlage toward the left, a condition resembling that just described for the salmon.

Migration of the Anlage Toward the Right

Göppert (4), Stöhr (17), and Stricker (18) showed that the original ventral liver anlage of the trout secondarily takes up a position to the right of the intestine. This was found to hold also for the salmon, except that the assumption of the dextral position was a very gradual process and not completely attained until shortly after hatching. Prior to that, most of the anlage lies on the right ventral surface of the intestine, the ductus choledochus entering its right lateral border.

Embryo: 6.5 mm., 14 Weeks, 140.0 Thermal Units

The embryo by continued growth has acquired the adult somite number of sixty. So far as external characteristics are concerned there is involved chiefly an increase in body size. The optic lobes are especially prominent but pigmentation is lacking. Through a secondary clockwise rotation or torsion of the long axis of the intestine when viewed from the anterior end, the anlage (Fig. 4) is gradually migrating from the left toward the right of the mid-line. This is accentuated as well by differential growth of the anlage itself, the posterior end now free of the intestine growing toward the right and anteriorly as a blunt projection. The maximum length of the anlage measuring from the entrance of the ductus choledochus posteriorly is 0.46 mm.

Embryo: 8.5 mm., 16 Weeks, 158.1 Thermal Units

Differentiation of the embryo is proceeding rapidly. The head is distinctly lifted off the yolk sac. The lens of the eye is commencing to develop crystalline fibres. A lumen has appeared in the pharynx, although the oesophagus is still occluded. The notochord is now composed entirely of vacuolated cells. The ventricular region of the heart has a conspicuous muscular development. The pectoral fins are large with a free thin terminal fringe.

The liver anlage (Fig. 5) has become hook-shaped, and has a maximal anterior–posterior length of 0.53 mm. The whole structure has migrated further toward the right but its left margin still extends over the corresponding margin of the intestine. The ductus choledochus enters the right lateral border of the intestine (Fig. 22). There is a cessation of rotation of the latter now until after hatching. The point of entrance of the ductus is 0.74 mm. from the posterior margin of the auditory vesicle and 0.11 mm. posterior to the anterior limit of the pronephric duct. The intestinal lumen is once more laterally flattened, although there is a tendency toward a horizontal shifting as indicated by its oblique position. The epithelial lining of the ductus is simple cubical or columnar, that of the intestine pseudostratified columnar. This type of epithelium is characteristic of the teleostean intestine (14, p. 174). The splanchnic mesoderm on either side of the intestine is heavy but becomes low and flattened ventrally as that from either side meets to form a ventral

mesentery between the ductus and the yolk. The epithelium of the intestine and ductus choledochus are covered externally by a thin connective tissue layer. The portion of the liver anlage lying immediately ventral to the intestine is still fused with the latter along the right ventrolateral surface, while that of the right anteriorly directed hook is free, a condition similar to that in the trout (18). Near the posterior end of the anlage (Fig. 23) the hepatic tissue measures about 0.4 mm. at its greatest diameter. The intestinal lumen is still somewhat oblique. The peritoneal epithelium has become flattened and scattered cells separate the ventral surface of the liver from the yolk. The hepatic cell cords are separated by connective tissue and numerous scattered vascular areas occur throughout the organ.

Subsequent Development of the Anlage

The liver remains in a right ventral position until shortly after hatching, but during this period is constantly shifting until it finally comes to lie entirely along the right margin of the intestine.

Embryo: 11.8 mm., 21 Weeks, 201.1 Thermal Units

In embryos of this stage the semicircular canals are commencing to develop in the ears. The oesophageal lumen is still occluded. Anteriorly the intestinal lumen is cylindrical, but posteriorly it usually becomes flattened dorsoventrally. The shape of the liver (Fig. 6) is not essentially different from that previously described, but it appears finely lobulated on the surface. It is somewhat rhomboidal in outline with one diagonal axis (0.80 mm. in length) passing parallel to the longitudinal axis of the intestine, the other transversely across the body at right angles. The cranial apex forms a hook on whose dorsal surface lies part of the pancreas and the ductus choledochus. The latter, arising from the liver, ascends rather abruptly and, passing transversely toward the left, discharges into the right lateral border of the intestine. At its point of entrance (Fig. 24) the intestinal wall is elevated to form the papilla duodeni which is at a level about 1 mm. posterior to the ear and projects 0.15 mm. into the lumen of the intestine. The pancreatic duct also empties into the papilla duodeni at a level slightly posterior to the opening of the ductus choledochus. On the dorsal surface of the liver the ductus choledochus is expanded into an elongated sac, the gall bladder. The intestine is lined with pseudostratified columnar epithelium with a cuticular border. It becomes simple columnar over the papilla duodeni and somewhat cubical in the ductus choledochus. The gall bladder is lined with cubical to low columnar epithelium. The cells of the liver are cubical with spherical nuclei and now appear to be arranged as branching trabeculae separated by large venous sinuses.

Embryo: 14.7 mm., 23 Weeks, 238.3 Thermal Units

Embryos at this stage possess melanic pigmentation in the choroid coat of the eye. Finely stellate melanophores are also appearing over the dorsal surface of the head and the trunk musculature. The pectoral fins are no

longer fleshy but thin and supported by rays. The gill slits are patent, but the oesophageal lumen is still occluded.

The liver (Fig. 7) is still somewhat rhomboidal, and covers the ventral surface of the intestine, but in most instances has a tendency to shift further toward the right. It is approximately 1.15 mm. in length. The intestine has acquired connective tissue and muscular coats about the epithelial lining, and a thin connective tissue coat also surrounds the epithelium of the gall bladder.

Embryo: 19 mm., 26 Weeks, 439.2 Thermal Units

Embryos at this stage of development have usually hatched, although not in this particular series. The body now forms a complete circle about the yolk sphere, the tail extending past the head sometimes as far as the margin of the operculum. Pelvic fins have appeared as fleshy buds at the posterior junction of the yolk sac with the ventral trunk musculature. The body axis lies with its right surface against the yolk, and the liver now occupies a position bordering on the yolk syncytium and almost entirely to the right of the digestive tract. The oesophagus is still occluded, and the anterior limit of the intestinal lumen is approximately the level of the anterior end of the pronephric duct. The right anterior tip of the liver appears 0.17 mm. in front of the level of the papilla duodeni which now projects about 0.18 mm. into the intestine. The papilla is covered by low columnar epithelium and possesses a connective tissue core surrounding the cubical to low columnar epithelium of the ductus choledochus. The liver has a maximum vertical depth of 0.9 mm. and a length of about 1.25 mm.

Alevin: 22.5 mm., St. John, 12 Days after Hatching

The alevin still has a heavy yolk sac. The eyes are deeply pigmented and scattered melanophores occur over the dorsal body surface. The dorsal and ventral fin folds are becoming differentiated. The gill slits are covered by the operculum. The digestive tract is still rectilinear (Fig. 8) and possesses a lumen except in the oesophageal regions. The liver has a sharply pointed right anterior projection, is broadest at mid-level and gradually tapers to its blunt posterior termination. It has a maximum length varying from 1.55 to 1.65 mm. and is situated on the right side of the intestine, curving slightly dorsal to it, and not infrequently may still project ventrally from the mid-level to the posterior end. The papilla duodeni (Fig. 25) enters the right side of the intestine about 0.4 mm. from the anterior tip, and now projects 0.2 mm. into the lumen. The liver at the level of the gall bladder and the first of the hepatic ducts, appears somewhat triangular in sectional area. Its dorsal surface is bounded by the body musculature, its left by the intestine, and the ventral by the yolk. Sinusoids occur between the trabeculae and larger veins are prominent about the gall bladder and duct.

Alevin: 25 mm., St. John, 24 Days after Hatching

At this stage the digestive tract is ceasing to be rectilinear (Fig. 9). In the adult salmon (Fig. 15) the oesophagus, cardiac stomach, pyloric stomach,

and ascending limb of the intestine (pyloric limb or duodenum) form an almost perfect *U*. From the pharynx, the oesophagus and cardiac stomach pass directly posterior for a distance equal to approximately one-half the length of the coelom. The tract then bends sharply on itself and passes directly anterior. The proximal half of this forward directed limb is the pyloric stomach, and the distal half, the ascending limb of the intestine, the pylorus marking the line of division between them. At the anterior end of the ascending limb of the intestine, another sharp flexure brings the small intestine directly posterior, that is, it forms the descending limb of the intestine which passes straight to the anus. Rotation at the pylorus has carried the descending limb of the intestine dorsolateral to the pyloric stomach and the ascending limb of the intestine. Pyloric caeca originate from the ascending limb of the intestine and from the region of flexure between it and the descending limb of the intestine. The initiation of the first or stomachal flexure in the digestive tract is apparent at the 25 mm. stage, somewhat anterior to the two primary rows of pyloric caeca anlagen. This flexion is the first indication of the division of the stomach into cardiac and pyloric limbs. The liver now lies wholly on the right side of the digestive tract and has a length of 1.6 to 1.7 mm. Its anterior extremity is sharply pointed. The left lateral margin appears somewhat irregular, the posterior is broad and blunt. Stricker (18) described in the larval stages of the trout, a somewhat rhomboidal-shaped liver with a right cranial hook comparable to that observed here in the salmon. He did not, however, carry development beyond the initial appearance of the first flexure in the digestive tract and through the subsequent migration of the papilla duodeni.

Alevin: 26 mm., St. John, 36 Days after Hatching

In this alevin the yolk sac has not noticeably decreased in extent except dorsolaterally, where its right lateral margin is now marked by an elongated ridge on the ventral surface of the liver (Fig. 10). Flexion of the digestive tract has increased and the ascending limb of the intestine has pushed forward against the right posterior surface of the liver. A transverse section at the level of the papilla duodeni (Fig. 26) passes directly through the intestinal flexure. The fact that the papilla duodeni has assumed a sinistrodorsal position is revealed. It is located now at the level of the union of the ascending and descending intestinal loops. At this time flexion of the intestinal tract is practically complete but torsion or rotation is just commencing. The displacement of the papilla duodeni through about 120° has occurred around the dorsal side of the intestine in a clockwise direction, if the tract be viewed from the anterior end. Simple flexion without torsion can account for this displacement.

Considerable histological differentiation is evident in the stomach and intestinal regions where the epithelial, connective tissue, and muscular layers typical of the adult have made their appearance. The liver cell trabeculae are more compactly arranged than in earlier stages.

Fry and Early Parr: 28, 30, 32 mm., St. John Hatchery, 1935

Further changes in the morphology of the liver occur simultaneously with yolk absorption and with the continued anterior growth of the ascending intestinal limb. The liver comes to occupy a position at the anterior end of the coelom just posterior to the septum transversum. In the 28 mm. fry (Fig. 11) the limit of the yolk sac is outlined on the lower surface of the liver. The posterior margin of the latter is gradually pushing toward the left and anteriorly immediately in front of the intestine. In addition to the original flexion, the gut has now undergone a torsion or rotation involving the pyloric stomach, the ascending and descending limbs of the intestine. As a result, the papilla duodeni has been rotated through 210° from its original position and now occupies a sinistroventral location on the ascending limb of the intestine immediately beyond the pylorus. The further displacement can only be attributed to about 90° of clockwise rotation of the tract when viewed from the anterior aspect.

At 30 mm. (Fig. 12) the yolk sac is absorbed with the exception of a triangular remnant adhering to the left lateral surface of the liver. The liver itself has assumed a somewhat triangular or cordate form, the former posterior margin now being directed to the left and posterior. The 32 mm. early parr (Fig. 13) possesses only a fragment of yolk at the left border of the liver. The latter is triangular in outline from the ventral view with the apex of the triangle directed toward the pylorus. The right lobe is growing posteriorly to cover the anterior part of the intestinal loop.

Liver–Yolk Relationship: St. John Hatchery Alevin, Fry, and Parr

The absorption of yolk is a major factor in the growth of the liver ventrally and toward the left from its early position to the right of the gut. Up to and including the 20 mm. alevin the liver lies directly on the dorsal surface of the yolk immediately to the right of the intestine. At 25 mm. a considerable quantity of yolk has been absorbed and the liver commences to grow ventrally from the right side toward the mid-line. The ventral growth continues and finally, disappearance of the yolk permits the liver to grow to the left and dorsally (31 mm.). It occupies most of the anterior end of the coelom about the stomach, the latter fitting into the gastric impression on its dorsal surface.

Development of the Biliary System

The relationship of the intestinal lumen to that of the biliary system has been illustrated by a series of graphic reconstructions of various developmental stages (Fig. 14, *a* to *i*). The intestinal lumen appears as a narrow irregular vertical slit at 3.8 mm. At 4.7 mm., when the anlage appears double in character, the lumen has become considerably deeper vertically. An embryo of 5.4 mm. possesses an irregular intestinal lumen which is joined anteriorly by the narrow ductus choledochus entering from the left side. The latter has an incomplete lumen which disappears as it reaches the anlage proper. By 6.5 mm. the anlage is shifting toward the right but the ductus still enters from the left. In the 8.5 mm. embryo through the clockwise rotation of the

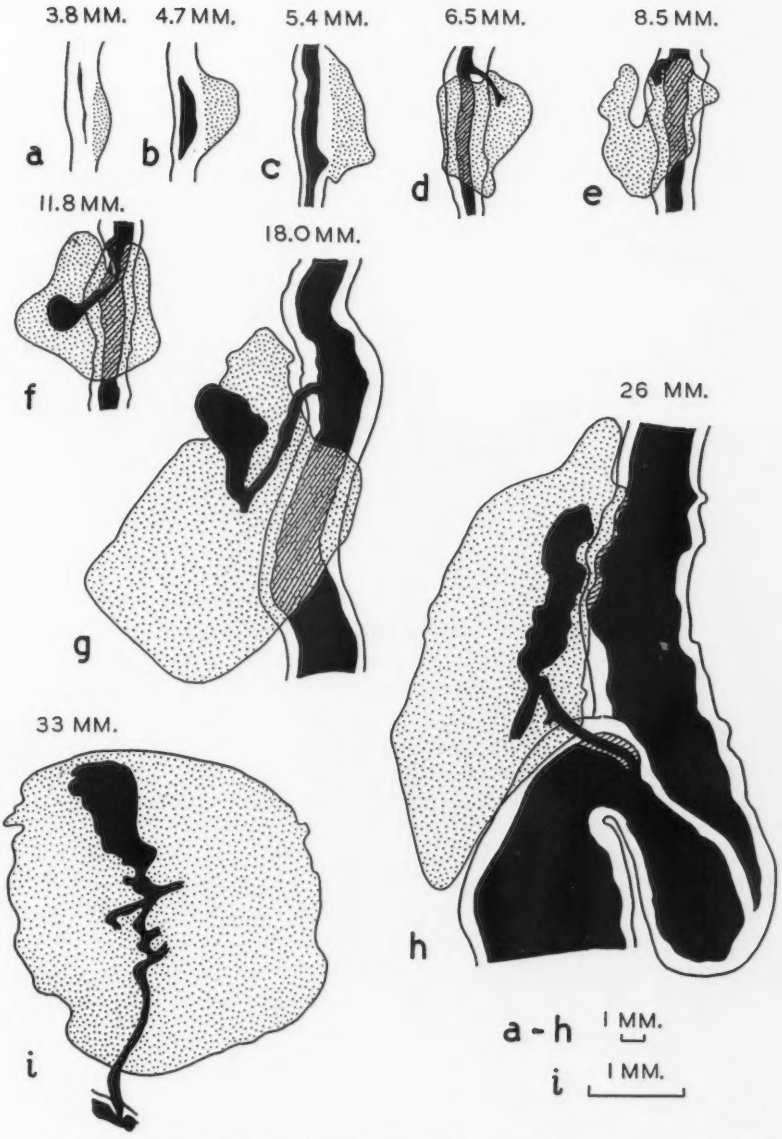


FIG. 14. *Graphic reconstruction of intestine (outline), liver (stippled), intestinal lumen and biliary system (solid black and shaded) in embryos, alevin, fry, and early parr at lengths of (a) 3.8 mm., (b) 4.7 mm., (c) 5.4 mm., (d) 6.5 mm., (e) 8.5 mm., (f) 11.8 nm., (g) 18.0 mm., (h) 26 mm., and (i) 33 mm.*

intestine, the ductus choledochus now joins the lumen of the latter from the right. The lumen of the ductus itself extends only from the intestine to the hepatic tissue. The first indication of the gall bladder is found in the 11.8 mm. embryo as an expansion of the posterior termination of the ductus choledochus on the dorsal surface of the liver. At 18 mm. the gall bladder has commenced to grow anteriorly thus forming an acute angle with the ductus. The first branch from the cystic duct is indicated passing into the liver parenchyma. Through flexion of the original rectilinear digestive tube, the liver is carried anteriorly in front of the intestinal loop (26 mm.). The ductus choledochus once more in linear relationship with the gall bladder enters the sinistrodorsal angle of the ascending limb of the intestine. At 33 mm. the liver, now occupying a position at the anterior end of the coelom, has on its dorsal surface anteriorly an elongated gall bladder, which drains into a curved cystic duct. The latter continues as the common bile duct, which receives several hepatic ducts from the liver substance. Torsion of the gut has caused its point of entrance to shift to the sinistroventral margin of the ascending limb of the intestine at the level of the second row of pyloric caeca.

Gross Morphology of the Liver

Parr and Smolt

After its migration to the anterior end of the coelom, the liver undergoes a gradual change in its general form (Fig. 15) through unequal growth toward the left. In the early parr it is somewhat triangular when viewed from the ventral surface, with the apex of the triangle directed caudad along the mid-ventral line (Fig. 15, *a* and *b*). Dorsally it is concave where it forms the gastric impression on the left, the intestinal impression on the right to conform with the contours of the cardiac stomach, and intestinal loop, respectively. Gradually the left half of the liver commences a posterior growth (Fig. 15, *c* to *f*). Prior to its transformation into a smolt, the liver of the late parr has already assumed the general proportions characteristic of the smolt (Fig. 15, *g* and *h*) and adult (Fig. 15*i*). This unequal growth of the liver may thus be considered as one of the changes accompanying the general metamorphosis of parr to smolt. The maximum anterior–posterior length of the liver in the parr is approximately one-tenth the body length. This proportion changes gradually until in the adult it is one-sixth.

FIG. 15. *Liver of representative parr and smolt from Nova Scotia and adults from St. John River, N.B.*

Parr: Ventral aspect, (a) 34 mm., Margaree River, August, 1935; (b) 40 mm., Moser River, August, 1939; (c) 54 mm., Moser River, August, 1939; (d) 63 mm., Moser River, August, 1939; (e) 158 mm., Margaree River, August, 1935; (f) 212 mm., Larry River, N.S., August, 1936.

Smolt: Ventral aspect, (g) 125 mm., Margaree River, June, 1938; (h) 220 mm., Lake Ainslee, August, 1935.

Adult: (i) Ventral aspect (92 cm. specimen) showing relationship of liver to adjacent viscera; (j) ventrolateral aspect (84 cm. specimen) showing posterior forking of left lobe and lappets; (k) dorsal, and (l) anterior aspect of same specimen as in (i).

Adult Salmon

The liver in the adult (Fig. 15, *i* to *l*) is a large yellowish- or greenish-gray to reddish-yellow gland situated at the anterior extremity of the body cavity. It is approximately one-sixth of the body length and imperfectly divided into

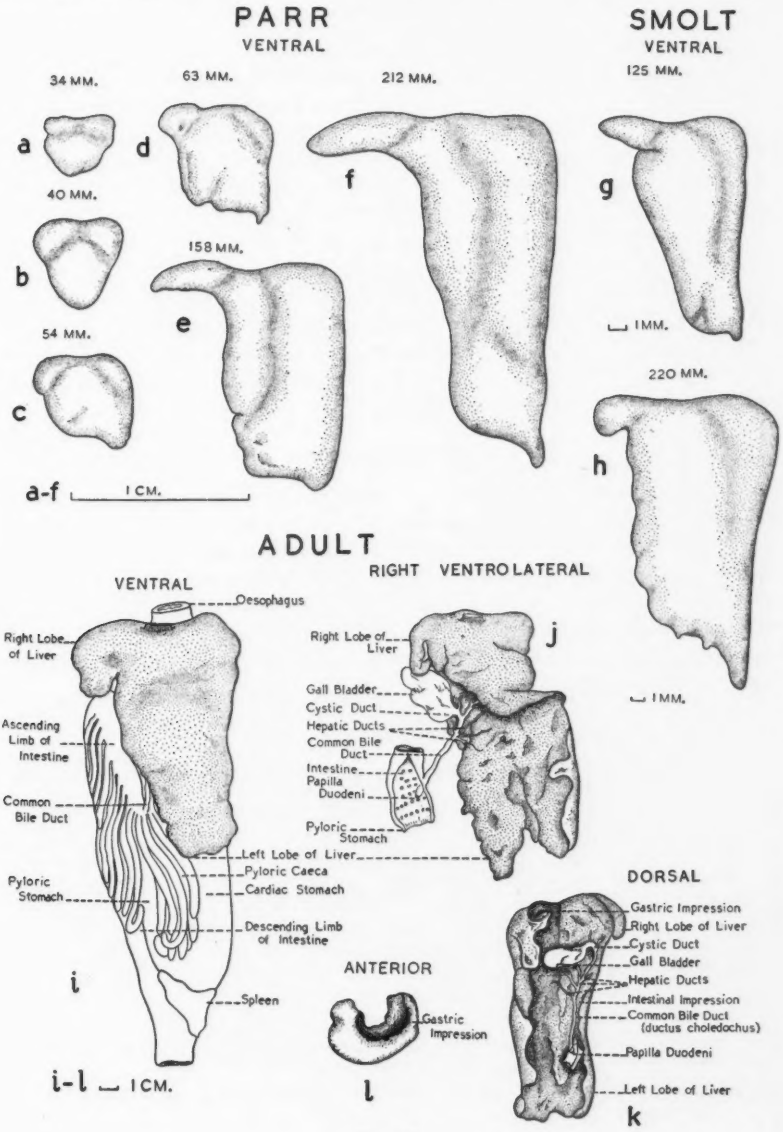


FIG. 15

a small right lobe and a larger left median lobe. The anterior and ventral surfaces are convex, while the dorsal or visceral surface fits the convexity of the stomach and the duodenum, providing the gastric and intestinal impressions. The organ is thick anteriorly but gradually becomes thin along the posterior and lateral margins which are composed of a number of dorsally flexed lappets similar to those described by Macallum (7) for *Ameiurus*. The right lobe is short, terminating immediately anterior to the intestinal flexure. The left lobe extends posteriorly over the stomach and the ascending limb of the intestine beyond the level of the entrance of the bile duct into the latter. The posterior margin of the lobe is not infrequently forked (Fig. 15*j*). The gall bladder is a somewhat conical or pear-shaped structure lodged in a fossa on the dorsal surface of the right lobe, with its blind end extending anteriorly and toward the left. Its long axis may extend transversely or diagonally from the gastric impression toward the right lobe where it passes into an arch-like cystic duct. The latter receives two or more ducts from the liver substance (hepatocystic ducts of Macallum (7)) and, in its posterior course, four or five hepatic ducts, whence it becomes the ductus choledochus (common bile duct). It enters the left ventral surface of the ascending limb of the intestine (duodenum) at the level of the second row of pyloric caeca, terminating at the tip of the papilla duodeni.

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